Symposium on

Steroids in Experimental and Clinical Practice

Edited for the Committee on Arrangements

by

ABRAHAM WHITE

1951

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Preface

In June, 1950, a group of individuals, later constituted as the Committee on Arrangements, was requested by I. V. Sollins of the Chemical Specialties Co., Inc., to discuss the feasibility of a conference dealing with steroids in experimental and clinical practice. Conversations among the Committee members led to general agreement that it would be of advantage to have the proposed conference evaluate the laboratory and clinical experiences with the 11-desoxy steroids, particularly inasmuch as several recent symposia and meetings had been concerned with the 11-oxysteroids, notably 11-dehydro-17-hydroxycorticosterone. Accordingly, plans were formulated which resulted in the First Annual Steroid Conference, held in Cuernavaca, Mexico, January 15–18, 1951. The invited speakers and guests were informed that the papers delivered at the conference, as well as the discussion, would be assembled for publication in book form. Each discussant was provided with the opportunity of editing his comments. Frequently, the discussion occurred following presentation of a group of papers, and in these instances the comments have been assembled following the last paper in the group.

The Committee on Arrangements requested the undersigned to function as editor of this volume. All of the material presented at the symposium has been included. There are a few differences between the order of the papers and discussions as printed and as presented orally; these alterations were made after discussion with, and approval by, the Committee on Arrangements. Little effort has been made to standardize the style of the manuscripts, particularly bibliographies, inasmuch as it seemed desirable to limit the extent of editorial changes in order to expedite prompt publication. In addition to the papers and discussion presented at the symposium, there are included five tables, which have been added at the end of the book. It was the opinion of the Committee on Arrangements that these tables might serve as a type of compound index, enabling the reader to ascertain quickly the salient information relating to the clinical investigations in this volume. In a sense, these tables represent a guide for summarizing the clinical studies presented at the symposium, and provide material from which the reader may formulate an opinion regarding the present status of the 11-desoxysteroids in clinical practice.

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April 1951

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1

Introduction to the Symposium

I. V. Sollins
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This Symposium on Steroids in Clinical and Experimental Practice will be, it is hoped, the first of a series of annual meetings established solely for the purpose of enabling and stimulating a free and unlimited exchange of information among clinicians, chemists, and biologists who are interested primarily in the general field of endocrinology—its chemistry, its physiology, and its clinical applications. With this thought in mind, the present symposium program in Cuernavaca was organized around the theme of the 11-desoxy steroids as a class.

Thus, the specific tasks of this first Cuernavaca conference are to study and ascertain the present status of 11-desoxy steroids in animals and man; to relate and integrate data obtained from various independent clinical and experimental studies concerning each individual steroid compound under investigation; to recommend further research along indicated lines with individual steroids or groups of compounds on the basis of the correlated data developed during these meetings; and to evaluate, if at all possible, the present or future clinical applications of such compounds. These tasks are not easily accomplished, for it is evident from the outset that existing knowledge concerning the pharmacology and metabolic or clinical activities of the 11-desoxy steroids is sparsely developed in some instances, heavily weighted by preconceived clinical opinions in others, and confused by seemingly contradictory information or inadequate studies in still other instances. Obviously not all these fragments of knowledge will be pieced together at this meeting, nor will all the confusion be dispelled. But at least the discussions to follow should produce some degree of agreement as to the nature of the problem, some common approach as to future investigations, and some immediately realizable although perhaps limited recommendations as to the clinical usefulness of many 11-desoxy steroids.

There is, of course, a substantial accumulation of knowledge concerning the classical sex hormones and desoxycorticosterone, all members of the 11-desoxy series; but of the hundreds of naturally occurring or newly created members of the 11-desoxy steroid family, almost nothing is known of their metabolic transformations or possible clinical applicability. This lack of knowledge concerning the 11-desoxy steroids applies

as well to all but one of the related 11-oxygenated compounds, namely, cortisone. However, here the dramatic impact of cortisone upon investigators and public alike plays a role. Without doubt, since the first announcement of cortisone's effectiveness in arthritis, there has been an overwhelming rush of activity on the part of investigators to study this 11-oxy steroid in scores of clinical or experimental conditions. More recently it has been realized that some of the other 11-oxy steroids, such as Compounds B, D, and F should be studied with equal intensity. Unfortunately, except in a few institutions, similar emphasis has until now not been given to the study of the 11-desoxy steroids, with the result that possibly beneficial or useful properties of some of these compounds may be overlooked. This conference can and should contribute the stimulus for intensive study of the hundreds of steroids in the 11-desoxy category. If it does this, it will have made a major contribution.

For the past two years, and more especially during the last year, the Syntex-Chemical Specialties organization which sponsored this meeting has very actively supported a program for the clinical and the biological investigation of the 11-desoxy compounds. The organic chemists of Syntex have synthesized literally hundreds of previously unknown compounds as well as scores of compounds that were previously known but until now available only in micro quantities. These steroids were distributed, without charge, in amounts ranging from a few grams to thousands of grams to investigators in many institutions for study in animal or in human subjects. There has always been only one restriction or stipulation, namely that these compounds be used and not be left sitting on laboratory shelves. Many investigators present here have benefited by this research program, and perhaps many more will benefit in the future, for it is intended to continue and extend such support to steroid investigation.

At this point it is perhaps appropriate to add that although the Syntex organic research program was for a time mainly concerned with the synthesis of 11-desoxy steroids from Mexican plant source materials, it has more recently also undertaken the synthesis of 11-oxy compounds from similar source materials. These studies have already resulted in the synthesis and production of several compounds in the 11-oxygenated series. In the near future some of these will be made available for research in the clinical field.

However, the Syntex group has in the past, and will continue in the future, to emphasize the advantages of the 11-desoxy compounds. They are available for clinical investigation in far greater quantities than are the 11-oxy steroids. They are far less costly to produce, since the vegetable sapogenins from which they are synthesized are abundant. Inasmuch as so little is known as to the possible clinical value of all steroids, it is indeed logical to investigate intensively those steroids that can be made available in large amounts, after their activity has been proved, rather than to concentrate research on compounds which presently or in the

foreseeable future can only be obtained in rare amounts and at uneconomic costs.

Perhaps one additional reason may be advanced for this present conference in Cuernavaca. It is highly essential to resolve some of the discrepant and often contradictory reports that have been obtained concerning various 11-desoxy steroids presently being utilized in clinical practice. For example, certain clinicians have observed and reported the value of pregnenolone in rheumatoid arthritis. Others either have not observed similar effectiveness or perhaps have employed different criteria in the measurement of clinical results. Perhaps conditions of treatment, such as dosages, routes of administration, length of treatment, or form of the compound have varied; the fact is that discrepant reports have appeared.

The same situation applies to reports concerning the clinical usefulness in arthritis of testosterone, other sex hormones, and some of the corticosteroids. Furthermore, similar problems also are present in evaluating steroid therapy in many other clinical entities ranging from collagen diseases to cancer. It is hoped, therefore, that such problems would be discussed adequately at this meeting and that suggestions would be forthcoming for means of resolving at least a few of these apparent differences.

The program of this conference provides for an emphasis on pregnenolone because this compound has been widely employed during the past year and because pregnenolone is a cheap, plentifully available, and perhaps clinically useful compound. However, the program proposes to raise various questions not only with respect to pregnenolone but also to other compounds about which conflicting or ambiguous clinical or biological data have been obtained. It is to be hoped that in the discussions and evaluations to follow, the members of this conference will attempt to equate data in terms of comparable units, i.e. patients of similar age, sex, and progression of disease; similar diagnostic standards and criteria of therapy; equal dosage levels for equal duration of therapy; similar routes of administration and similar pharmaceutical dosage formulations; as well as many other factors that govern an experiment, whether it is clinical or biologic. There would seem to be rather obvious precautions to employ in objective evaluation of new drugs, and yet perhaps because of their obviousness it is not surprising to find that much of the published literature pertaining to the subject shows the absence or disregard of these equating considerations. There has been a marked tendency for certain investigators to write off a number of steroids as completely useless on the sole basis of inadequate comparisons. This conference can correct such mistakes.

When the idea of this meeting first arose, some six or eight months ago, the counsel of Drs. Reifenstein and Pincus was sought. After preliminary conversations with and advice from these workers, an Advisory or Arrangements Committee was organized from among scientific workers in Mexico and the United States. This committee planned, or at least counseled in the planning, of the program that now constitutes the

agenda of this conference. What is to be presented here at Cuernavaca for discussion and consideration of the conference members, therefore, represents the best efforts of the committee who have so kindly helped in the organization of the meeting.

Most sincere thanks are extended to the members of the committee and to the participants in this conference who have taken valuable time from their daily work to visit Mexico for this meeting.

2

The Physiology and Pharmacology of the 11-Desoxy Steroids

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In the short space of a single lecture, it would obviously be impossible to present an exhaustive account of the pharmacology of the 11-desoxy steroids. We thought, therefore, that it would serve the purposes of this conference best if, after some introductory remarks concerning the definition of our terms, we devoted special attention to three compounds of this group: Δ^5-pregnenolone, 21-acetoxypregnenolone, and desoxycortisone. These were singled out merely because we happen to have more personal experience with them than with most of the other 11-desoxy compounds which may deserve equal attention. It is true that my co-workers and I have done more work with desoxycorticosterone than with any other hormone. However, I shall not speak about this steroid today except insofar as it illustrates the pharmacology of the three compounds selected for special discussion, because its properties are already quite well known. The reader interested in a more detailed discussion of the pharmacology of other steroids is referred to several pertinent monographs in which we dealt with this subject.[1, 2, 3]

Finally, we shall say a few words about the correlations between anterior pituitary hormones and the 11-desoxy steroids, since recent observations made in our Institute suggest interesting new possibilities for research in this field, especially in connection with what we have termed "the diseases of adaptation."

Definition of Terms

The basic principle according to which the pharmacologic activities of steroids are classified is that certain actions are independent of each other while others are merely the subordinate manifestations of such independent actions, and hence are dependent upon them. It must be clearly understood that independent actions are characterized by the fact that every one of them can be exhibited independently of any of the others; that is to say, there is no direct parallelism between the degrees to which a compound exhibits the various independent actions. In this sense we recognize the independent nature of the following actions:

1. *Folliculoid* (estrogenic, gynecogenic, estromimetic, or follicular hormone-like).

2. *Testoid* (androgenic, andromimetic, or male hormone-like).

3. *Luteoid* (progestational, corpus luteum hormone-like).

4. *Corticoid* (adrenal-cortical hormone-like).

5. *Spermatogenic* (having the ability to stimulate the spermatogenic epithelium and mainly to protect it against atrophy caused by deficiency in gonadotrophic hypophysoid hormones).

6. *Renotrophic* (nephrotrophic, having the ability to increase kidney size due mainly to hypertrophy of the convoluted tubules).

7. *Anesthetic.*

8. *Antifibromatogenic* (inhibiting fibroma formation by folliculoids).

It will be noted that for those independent actions which imitate the function of an organ of internal secretion, terms are used which suggest a specific connection with that particular endocrine gland. The Greek ending "-oid" which is added to the name of a gland means "similar to" without implying that the hormone is necessarily derived from that particular gland. It merely suggests that the compound simulates the gland's activity, and this is true by definition. It would be misleading, for instance, to designate hormones of the testoid type as "testicular hormones" since such compounds are also elaborated by the adrenal cortex and probably even by ovarian and placental tissues. It would be equally misleading to term them "androgenic" since the most potent natural "androgen," testosterone, causes testis atrophy in experimental animals. Thus, far from being masculinizing, it is actually "demasculinizing." Similarly, in many animal species, the so-called "estrogens" do not in themselves cause estrus or heat without simultaneous progesterone treatment, hence the latter hormone could be called "estrogenic" with almost equal justification. Furthermore, folliculoids interrupt the estrous cycle in the intact rodent so that they are actually "antiestrogenic" under ordinary circumstances of bioassay. The term "gynecogenic" is no more adequate for them since they cause ovarian atrophy.

In the light of the above considerations it appears unsatisfactory to designate these hormones either according to their source of origin or on the basis of one particular action on a certain target organ. It is for these reasons that we give preference to the terminology which is based upon the natural classification of the independent steroid hormone actions according to the gland whose function they simulate.[4]

Δ^5-Pregnenolone

The pharmacology of Δ^5-pregnenolone has recently been excellently and exhaustively reviewed by Henderson, Weinberg, and Wright,[5] so that we shall limit ourselves to some personal observations.

It is of historic interest that although this compound had been prepared as early as 1934,[6] for many years it was not considered to have any physiologic or pharmacologic activity; it was used merely as an interme-

diary in the synthesis of other steroids. Several investigators examined it for possible folliculoid, corticoid, or luteoid potency,[6, 7, 8] but concluded that it was inert, because they gave the compound at ineffective dose levels.

Probably because the folliculoids and the testoids happened to be the first steroid compounds available for pharmacologic and clinical study the impression became strongly ingrained among students of this field that hormonal steroids are always effective in minute dosages. It is now obvious, however, that in order to exert their full effect, folliculoids (and to a lesser extent testoids) are weight for weight much more potent than most of the other hormonal steroids. Until we learn more about the absolute quantities at which these compounds are secreted by the endocrine glands under normal and pathologic conditions, it would be difficult to designate any dose as "unphysiologic" unless it approaches a toxic or lethal level.

It was this consideration that led us to experiment with comparatively high doses of pregnenolone and to the finding that this compound possesses a high degree of spermatogenic activity; it protects the seminal epithelium against the involution normally induced by hypophysectomy or folliculoid overdosage. It was subsequently noticed that pregnenolone also possesses some degree of luteoid, corticoid, folliculoid, anesthetic, renotrophic, and, in a sense, testoid activity. The latter effect is somewhat doubtful since pregnenolone stimulates only the prostate and the preputial glands, not the seminal vesicles, of the castrate rat.[9]

In view of these pharmacologic activities, Ruzicka[10] and later Haines and his associates[11] attempted the identification of pregnenolone in testicular tissue and found it present in appreciable amounts in hog testes. This suggests that pregnenolone is a true testicular hormone. Of course, for the final proof it would have to be shown that the substance is actually secreted into the blood, but we must not forget that many substances which we generally regard as authentic natural hormones have never been definitely demonstrated in the circulation under normal conditions (e.g. parathyroid hormone, prolactin, ACTH, etc.).

If the steroid hormones are derived from cholesterol, pregnenolone would be a logical parent substance of all steroid hormones, as it differs from cholesterol only in the side chain. This fact and the manifold, though slight, pharmacologic actions of pregnenolone led us to consider the possibility "that the specialization of a certain pharmacological action occurs gradually and always at the expense of other activities which are present—even if only to a slight degree—in the parent compound. This differentiation is rather reminiscent of the evolution of highly specialized cells from 'multipotent' but undifferentiated embryonic cells."[1]

21-Acetoxypregnenolone

21-Acetoxypregnenolone is much more active than pregnenolone as regards its corticoid effects.[12-16] It also possesses some anesthetic and luteoid activity as well as a trace of folliculoid potency, but appears to be entirely devoid of testoid actions.[1, 2]

The chemical relationship between pregnenolone and 21-acetoxypregnenolone is the same as that between progesterone and desoxycorticosterone acetate, since in both pairs of compounds the last-mentioned compounds differ from the first compounds only by the introduction of an acetoxy grouping at C_{21}. Interestingly, in both these cases, luteoid activity is diminished while corticoid potency is augmented by the introduction of this acetoxy group.

Up to the present it has not been possible to demonstrate the occurrence of 21-acetoxypregnenolone or the corresponding nonacetylated compounds in the body under normal conditions.

Desoxycortisone

Desoxycortisone (also known as "Reichstein's Compound S") differs from cortisone only in that it does not possess an oxygen at C_{11}. Its presence in the adrenal cortex has repeatedly been demonstrated by actual isolation and the compound has been shown to possess a comparatively high degree of mineralo-corticoid potency.[2]

This steroid has been of special interest to us because of its great pharmacologic similarity to desoxycorticosterone. The production by the latter compound of nephrosclerosis, hypertension, periarteritis nodosa, and myocarditis similar to that seen in rheumatic fever has been considered for a long time by various investigators, to be of purely toxicologic interest, since the existence of desoxycorticosterone in the adrenal gland was subject to doubt.

It will be recalled that desoxycorticosterone exerts its above-mentioned pathogenic actions most readily in animals sensitized to these "mineralo-corticoid effects" by unilateral nephrectomy and a high sodium intake.[17] As soon as adequate amounts of desoxycortisone became available to us, we therefore examined its activity under similar conditions of sensitization and found that it shares all the above-mentioned properties of desoxycorticosterone.[4, 18] Since there can be no doubt about the normal occurrence of desoxycortisone in the adrenals, we may safely conclude that natural mineralo-corticoids do possess toxic effects upon the kidney and the cardiovascular system. Recent observations of the Worcester group,[19] in which an ingenious *in vitro* perfusion technique was used, have furnished definite evidence that desoxycorticosterone also occurs in the adrenal gland and is actually secreted into its veins.

On the basis of the above-mentioned observations it appears highly probable that desoxycortisone and desoxycorticosterone, like "mineralo-corticoid" compounds, play an important role in physiology and pathology. Hence, further investigations into the intimate mechanism of their actions would be highly desirable.

Interactions Between the Anterior Pituitary and the 11-Desoxy Steroids

Some time ago, we observed that crude anterior pituitary extracts[20] or lyophilized anterior pituitary tissue (LAP)[21] duplicate the above-

mentioned actions of mineralo-corticoids upon the cardiovascular system, the blood pressure, and the kidneys. The hypophyseal preparations which we used were definitely corticotrophic in that they enlarged the adrenal cortex, but they also possessed marked "growth hormone" or somatotrophic hormone (STH) activity.

As soon as we were able to obtain purified ACTH it became evident that the above-mentioned pathogenic actions of the crude anterior pituitary preparations could not be due to their ACTH content, since even the highest tolerable doses of the latter hormone fail to duplicate these effects. On the other hand, overdosage with pure STH caused cardiovascular and renal lesions identical with those previously observed in animals treated with mineralo-corticoids. It was concluded that the above-mentioned actions of our crude anterior-pituitary preparations were due to their STH content.[22] It remains to be seen to what extent STH acts directly, by stimulating the mineralo-corticoid production of the adrenal cortex, or indirectly, by sensitizing the peripheral tissues to the mineralo-corticoids. Preliminary observations suggest that both these mechanisms are implicated.

It has also been found that STH greatly sensitizes the rat to threshold doses of desoxycorticosterone. Combined treatment with these two hormones within 10 to 12 days causes markedly pronounced cardiovascular and renal damage with diabetes insipidus-like polyuria in the rat. This results in a progressive and eventually fatal hypertension, even if the hormone treatment is discontinued.

It would be highly desirable to repeat these experiments with desoxycortisone and other naturally occurring 11-desoxy steroids.

A few years ago, we found that pregnenolone, which in itself possesses only a mild stimulating effect on the preputial glands, causes an extraordinary hypertrophy and hyperplasia of these glands when it is given concurrently with doses of LAP which, in themselves, are almost ineffective.[9]

Recently, we observed that STH likewise possesses a slight stimulating effect on the preputial glands, just as LAP does. We have not yet had occasion to test the influence of pregnenolone upon this effect, but our experiments have already revealed that concurrent administration of cortisone greatly augments the hypertrophy of the preputial glands caused by STH. The corticoids produced under the influence of ACTH appear to duplicate the action of cortisone in this respect, since simultaneous treatment with ACTH and STH likewise results in a particularly pronounced enlargement of the preputial glands.

While the preputial glands are not of particular physiologic or medical significance, they may act as highly sensitive indicators of STH potency, especially when the latter is given in conjunction with a variety of steroids. Interestingly, simultaneous treatment with LAP and desoxycorticosterone[9] or STH and desoxycorticosterone[23] did not result in any increase of the preputial glands above that obtained by LAP or STH alone.

In respect to this augmentation effect there appears to be an essential similarity between pregnenolone and cortisone and a fundamental difference between pregnenolone and desoxycorticosterone. Of course, various testoid compounds stimulate the preputial glands and can augment the corresponding action of LAP,[9] but since neither cortisone nor desoxycorticosterone have any demonstrable testoid activity, this does not appear to be a prerequisite for the above effects.

Another rather important—and perhaps somewhat neglected—field of investigation concerning the interactions between the anterior pituitary and the 11-desoxy steroids is the ability of many members of the latter group to diminish ACTH production and thus to cause adrenocortical atrophy. It is well known that cortisone, and to a lesser extent desoxycorticosterone, cause adrenocortical atrophy through this mechanism,[4] but, curiously enough, testosterone and various other androstane derivatives share this property. This is especially noteworthy since—contrary to current opinion—testoids are even more effective than desoxycorticosterone in inhibiting the adrenal enlargement normally observed during the alarm reaction. The intensity of their anticorticotrophic effect was demonstrated in rats in which systemic stress was produced either by formaldehyde injections[24] or by very high doses of thyroxine.[25, 26]

A systematic study of the anticorticotrophic effect of various 11-desoxy steroids would appear to be promising in connection with various clinical syndromes resulting from hypercorticoidism.

The diagrammatic chart (Fig. 1) will help to visualize interrelations between the pituitary and the adrenal cortex during stress. Although the precise mechanism of the STH action is not yet fully ascertained, it appears to be most readily compatible with this interpretation.

The *stressor* (trauma, infection, burns, etc.) acting directly upon the cells produces *damage*. At the same time, it also mobilizes *defense* by evoking a stimulus, which induces the anterior pituitary to produce ACTH; under certain circumstances it may also cause a discharge of STH. The nature of this first mediator between the directly injured organ and the anterior pituitary is not yet known (humoral, nervous?). Hence, here it is indicated merely by an interrupted line, labeled with a question mark. *ACTH* induces the adrenal cortex to produce predominantly *glucocorticoid* compounds, whose effect upon the response of the various target organs is generally inhibitory (e.g. catabolism, and diminution of granuloma formation and of allergic responses). Conversely, STH enhances a variety of defensive reactions in the target organs (e.g. anabolism, and augmentation of granuloma formation and of allergic responses), primarily by stimulating the connective tissue. Part of this action is undoubtedly not mediated through the adrenal cortex, and this direct effect sensitizes the connective tissue elements to the (essentially similar) actions of the *mineralo-corticoids*. It is probable that STH also acts by increasing the production of mineralo-corticoids. However, in itself, it cannot maintain the cortical cells in a responsive condition, hence its "corticotrophic" effect is dependent upon the simultaneous availability of ACTH. In the

final analysis the physiologic and pathologic responses of the target organs to stressor agents largely depend upon the balance between the mineralo-corticoids and STH on the one hand, and ACTH and the gluco-corticoids on the other.

FIG. 1. Schematic diagram illustrating the principal interrelations between the hypophysis, the adrenal cortex, and the peripheral target organs during the general-adaptation-syndrome. (Slightly modified after H. Selye, "First Annual Report on STRESS," Acta Inc. Med. Publ., Montreal, 1951.)

Summary

After a brief consideration of the classification of steroid hormone actions, there has been surveyed in more detail some of the most interesting properties of Δ^5-pregnenolone, 21-acetoxypregnenolone, and desoxycortisone (Reichstein's Compound S).

Special consideration has also been given to the interactions between the anterior pituitary and the 11-desoxy steroids, and promising avenues for future research along these lines have been outlined.

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Discussion

K. Dobriner: I am very interested in Dr. Selye's remarks, especially in relation to the role of the growth hormone in adrenal physiology. Is there any experimental evidence for the direct action of the growth hormone on the adrenal gland? Could there be a synergistic effect of growth hormone on the adrenal gland? There could be a synergism of growth hormone and hormones of the adrenal gland without direct action of the growth hormone on the adrenal.

I am in agreement with Dr. Selye that Compound S is an important adrenal hormone. We have isolated relatively large amounts of this hormone from quickly frozen hog adrenals, and I believe that Compound S constitutes a significant fraction of the steroid secreted by the adrenal. I believe that 11-desoxy steroids contribute significantly to the urinary steroid excretions.

H. Selye: Dr. Dobriner wanted me to give evidence for the participation of the adrenal in the STH effects which I have illustrated here. The most important part of the evidence in this connection is that if you adrenalectomize the animals, as I said, then STH plus ACTH (a trace of ACTH

is always necessary to get the STH effect, as indicated in the chart of my paper) no longer produce any "collagen disease" or any of the other lesions which I have described. Animal experiments in which no hormone determinations are made cannot be used as definite evidence in favor of the concept that STH acts by increasing the mineralo-corticoid (M-C) production. That is why in my detailed drawing in the paper I had one branch of the STH arrow pointing to the target organ directly. There is no doubt about the correctness of this branch. There is certainly a direct effect of STH on connective tissue. Very probably STH and mineralo-corticoid compounds synergize each other in the periphery. Whether STH also acts to increase corticoid production we cannot say with certainty until steroid metabolism studies have given us some more precise indication. The fact that adrenalectomy prevents these STH effects could well be due to the elimination of those endogenous corticoids, which are necessary for the peripheral sensitization of the connective tissue to STH. In the final analysis, for the interpretation of what we call "collagen disease," it does not matter very much whether STH acts through, or in conjunction with, mineralo-corticoids, as long as we realize that STH causes mineralo-corticoid effects. The most important conclusion which I think should be made on the basis of this work is that STH is almost the exact counterpart of ACTH. Perhaps instead of saying that STH is a "mineralo-corticotrophic hormone," it would be more prudent in the meantime to speak of it as a "mineralo-corticoidomimetic" hormone—to indicate that it imitates the mineralo-corticoid effects—but this is a terribly cumbersome term.

A. White: Could one actually settle the point of difference that has been raised with respect to the role of the adrenal cortex by studying the effect of STH in an adrenalectomized animal maintained with suboptimal amounts of adrenal cortical extract to produce the so-called tissue sensitization? Under these circumstances, extra corticoid or desoxycorticoid production by the adrenal would not be a significant factor.

H. Selye: We have already done that experiment and it yielded a positive result, so that there is no doubt about the peripheral synergism between the two hormones. The only question is whether, in addition to this, STH also augments mineralo-corticoid production.

F. Homburger: I think we can add to the eight independent actions of steroid substances a ninth one which was originally described by Dr. Selye, namely the ability to protect the renal epithelium in the experimental hydronephrotic kidney. We believe that this is independent from the simple renotrophic effect for the following reasons. With testosterone, for instance, this effect in the castrate female mouse is completely counteracted by stilbestrol, whereas the classical renotrophic effect is not. Further, if one screens a number of renotrophic and epithelium protecting compounds, one finds differences in degree of these two phenomena. Also,

certain progesterone derivatives will not have the renotrophic effect unless estrogen priming is first done in the castrate mouse but they do have the epithelial protective effect without priming.

J. W. Conn: I think Dr. Selye has intentionally, perhaps, tried to oversimplify the problem of antagonism between the so-called mineralo-corticoids and gluco-corticoids. There is much overlapping in the physiologic properties of many of these compounds as they affect both inorganic and organic metabolism. When ACTH is given to man, there results not only the metabolic effects of gluco-corticoids, but also very dramatic and marked mineralo-corticoid effects as well. Thus, one gets very definite desoxycorticosterone-like effects upon administration of ACTH to man, as well as many cortisone-like effects.

Because a number of pure steroid compounds have been shown, when administered to man, to induce different degrees of metabolic activities in these two broad phases of metabolism, the point of view has been taken by many that the adrenal cortex actually makes these various compounds and that the overall metabolic effect of cortical stimulation represents the resultant competitive and additive effects of many steroids acting peripherally. While this thesis may prove to be correct, it is not a necessary conclusion in the light of our present knowledge. Corticosterone or Compound B, which we have recently studied intensively, produces qualitatively all of the metabolic effects which are observed when a combination of DCA and cortisone is administered. Thus, the possibility still exists that the adrenal cortex actually produces only one or two physiologically active steroids, and that depending upon the receptivity (conditioning circumstances) of the peripheral tissues, the metabolic results of these steroids will vary profoundly.

H. Selye: I am very glad Dr. Conn brought out these details, because in the very short time at my disposal within the framework of my lecture I couldn't have gone into this adequately. I certainly agree that there is no such thing as a *pure* gluco-corticoid compound. This is not without precedent in steroid pharmacology. There is no such thing as a pure testoid (or androgenic) compound either. All testoids have some detectable folliculoid (estrogenic) effects if you test for them under suitable conditions. Most of the steroids have several "independent" actions. That does not detract from the value of the classification of steroid hormone effects on the basis of the independent actions. On the contrary, it helps us to make our terms more precise. If one wants to say pure gluco-corticoid effect, one should not speak of "cortisone-like action" because cortisone itself is not a pure gluco-corticoid. The gluco-corticoid effect is but one action of certain steroids. Pure gluco-corticoid compounds have never been described. There may be more or less of it in a mixture of other activities in individual chemical compounds. DCA is probably the prototype of the mineralo-corticoid compound; it has little or no gluco-corticoid effect, but it does possess some luteoid (progestational) potency. Cortisone and

"Compound F" are typical gluco-corticoids, yet they also have mineralo-corticoid actions. I think it is better to talk of gluco-corticoid and mineralo-corticoid *actions* rather than *compounds.*

As far as ACTH is concerned, it certainly also has some mineralo-corticoid effect; you can get sodium retention and water retention with ACTH. The question is whether this mineralo-corticoid action is due to the production of predominantly mineralo-corticoid compounds, or to gluco-corticoid steroids which also happen to have some mineralo-corticoid effect. If ACTH produced cortisone only, it would still have some mineralo-corticoid effect because cortisone itself has them.

Dr. Conn pointed to the very important factor of the sensitivity of the peripheral target organ (the peripheral cells) to the hormone actions. It is in this sense, I think, that a direct action of STH upon the connective tissue should be considered. As I said, the most clearly established portion of the work on STH reported here is that this hormone considerably increases the mineralo-corticoid type of effect of steroids *in the periphery.*

A. Hellbaum: I would like to ask Dr. Selye to indicate where the adrenal androgenic or ketosteroid excretion fits into the pituitary-adrenal relationship he has pictured.

H. Selye: As regards the stimulation of androgen production of the adrenals, I couldn't report on any personal experiments. I think the question of a special hormone being in charge of this androgen secretion must be studied. But it is also possible that, depending upon incidental conditions, ACTH might do that. I don't think that question can be answered definitely today.

A. Segaloff: I would like to comment on the assay of different types of activity. There can be qualitative differences in activities depending upon the test object chosen. We have been interested in the comparison between glycogenic activity in adrenalectomized animals and the ability of the same steroid to produce glyconeogenesis in adrenalectomized animals given phloridzin. In the phloridzinized animal, cortisone is rather poor in causing glyconeogenesis, being no better than 11-desoxycorticosterone. By the method of glycogen deposition you get very large amounts of glycogen with cortisone and either none or very little with 11-desoxycorticosterone. Compound S, at least in our hands, has given us glyconeogenesis in neither of the test objects. It appears that in thinking about glyconeogenesis, the tissue sensitivity which has already been mentioned is of the utmost importance.

Dr. Selye stated that at least in large amounts, Δ^5-pregnenolone will maintain the adrenalectomized animal. What does he consider are large amounts?

H. Selye: As regards the corticoid activity of pregnenolone, I was referring to experiments performed on adrenalectomized rats, which were not

given extra sodium and were receiving about 10 mg. of pregnenolone per day. The life maintenance was thereby increased over that of the untreated controls. The corticoid activity of pregnenolone is extremely slight, even with very high doses, and even progesterone has a greater corticoid activity than pregnenolone.

R. G. Sprague: Dr. Selye's presentation was broad in scope and he obviously could not give all the evidence in support of some of the points which were made. For example, his point that growth hormone has adrenotrophic activity would be greatly strengthened if it could be shown that a highly purified preparation of this hormone is capable of maintaining or increasing the weight of the adrenals of the hypophysectomized animal. Is such evidence available?

The statement that Compound S has appreciable DCA-like activity has been made and has been widely quoted. What is the evidence in support of this in terms of effects of Compound S on electrolyte balance? The evidence from studies of human subjects with which I am familiar indicates that it does not have much DCA-like activity.

H. Selye: As regards the morphological effect of STH upon the adrenals, C. H. Li found that the adrenals of hypophysectomized rats are not, or only slightly, stimulated by pure STH in the dosage used. In animals in which an adrenal atrophy has been produced by cortisone, STH tends to antagonize that atrophy, but only at relatively high dose levels. If STH is given to an intact animal (not hypophysectomized or otherwise treated), it causes a considerable enlargement of the adrenals and these adrenals are always very rich in lipids. It is quite conceivable that STH does this because it elicits a compensatory ACTH discharge. This wouldn't be without precedent in endocrinology, because if you give FSH to an animal with its pituitary intact, you will immediately get an LH secretion and vice versa. The two gonadotrophic hormones are discharged to complement each other; whenever one is introduced in excessive amounts, the other is discharged, and that may be true of the relationship between STH and ACTH.

As regards the evidence for the mineralo-corticoid activity of Compound S, we have the following observations. First of all, Compound S in adequate doses (in sensitized animals) will produce periarteritis nodosa, myocarditis nodules, and nephrosclerosis with hypertension, just like DCA. It produces marked polyuria and water retention. The production of edema and polyuria are certainly an effect on water and electrolyte metabolism and the morphological changes are precisely the same as those produced by DCA and other substances with mineralo-corticoid activity. With the additional Compound S which we have now received from our host we will be able to do more relevant mineral-metabolism studies.

R. Hertz: Does this effect of STH on the adrenals have anything to do

with its demonstrated diabetogenic action which has now been reported from two laboratories?

H. Selye: We have no evidence that there is any relationship between the diabetogenic action and the mineralo-corticoid action of STH. Under the conditions of our experiments it did not produce any diabetes and it did not produce any histological evidence of damage in the Langerhans islets of the pancreas. Since the question of the diabetic action of STH has been brought up I might mention that this effect can be effectively counteracted by giving insulin at the same time. Anyone wanting to use STH in clinical medicine should bear this inhibiting effect of insulin in mind.

A. White: Dr. Conn raised a point and Dr. Selye agreed, I believe, that one should think of these actions of steroids not in terms of a rigid classification but recognize that overlapping of physiologic actions does exist. Dr. Selye also suggested that in some of the clinical circumstances in which ACTH and cortisone were contraindicated, STH or growth hormone, in combination with one of the desoxy steroids, might be indicated. I would like to ask, therefore, whether or not it is possible that in some of the instances where the desoxy steroids have been reported to have some therapeutic effect, this effect might be enhanced by the administration of STH at the same time?

H. Selye: As regards the effect on connective tissue structures and connective tissue reactions (particularly inflammatory reactions) our experimental evidence is based mainly on irritation of connective tissue with phlogistic agents. For instance, mustard powder or formaldehyde will cause periarthritic inflammation if injected in the vicinity of joints. This inflammatory reaction is very effectively inhibited by cortisone and ACTH. In our experience in this type of experiment with the rat, and with these particular irritants, we did not find any 11-desoxy compound with a therapeutic effect. On the contrary, desoxycorticosterone and STH had a definitely unfavorable effect. They increased fibroplasia, granuloma formation, stiffening of the joints, and so forth. I do not think that the future of 11-desoxy compounds lies in trying to imitate cortisone actions with them. It is more likely that these 11-desoxycorticoids have a use when granulosa formation is advantageous, as for instance in non-union fractures or in tuberculosis, where spreading is to be prevented by fibroplasia and encapsulation. If you heavily overdose animals with cortisone, one of the common causes of death is the development of pulmonary or renal abscesses. I have never yet seen such abscesses in an animal given cortisone with STH.

The idea of a synergism with STH, as Dr. White expressed it, seems to have good possibilities. I gave one example of this as regards the effect of STH plus pregnenolone upon the preputial glands. Other such synergisms between STH and 11-desoxy steroids may exist.

A. White: For purposes of the completeness of the record, Dr. Selye, would you state which desoxy steroids you have studied which exhibit this capacity to produce, under suitable conditions, a variety of histologic lesions? Is your experience limited to desoxycorticosterone and Compound S? Can one say, on the basis of studies with desoxycorticosterone and Compound S, that all desoxy steroids would have a like action?

H. Selye: The "hyalinosis syndrome," or "collagen disease syndrome," has now been produced in the rat by DCA, desoxycortisone, and STH. I haven't been able to produce it with any other desoxy steroids in the rat. The chick is the animal in which the whole hormonal hyalinosis syndrome was originally discovered because it is extremely sensitive to these hyalin changes. In chicks acetoxypregnenolone and even large doses of progesterone have also had such an effect. It is conceivable that it could be produced with other desoxy steroids if the animal were sensitized with STH. Incidentally, I think it would be wrong to look at this as an entirely undesirable action of steroids. Heavy overdosage with any hormone will cause trouble. We used these lesions for the study of the role of the adrenal in hypertensive and rheumatic diseases. In that sense it was necessary to show that mineralo-corticoid compounds can produce these lesions, but I think in smaller doses, and under different conditions of hormone administration, the stimulation of connective tissue repair and the activation of the inflammatory potential of connective tissue could also be used to advantage.

T. H. McGavack: Is there any available evidence regarding a possible influence of 11-desoxy corticoids upon the secretion of STH?

H. Selye: Not to my knowledge.

W. Bauer: I wish Dr. Selye would explain why the administration of desoxycorticosterone to patients with rheumatoid arthritis does not aggravate the disease. To date we have never observed an exacerbation of the disease process during the administration of either desoxycorticosterone acetate or the glycoside, even when considerable salt retention occurs. Furthermore, we have found that cortisone is equally as effective when administered simultaneously with desoxycorticosterone as when administered alone.

H. Selye: I am afraid I don't have any answer to that question. I remember that at one of the Macy Conferences Dr. Kendall gave us some information on that and perhaps Dr. Sprague could answer your question.

R. G. Sprague: I am not familiar with that evidence.

H. Selye: I know that several investigators have reported joint lesions in Addisonian patients treated with DCA. Why it does not aggravate at the dose levels given I don't know.

G. Sayers: I am glad to hear that Dr. Selye no longer considers the adrenocorticotrophic hormone as the factor responsible for the induction of pathologic changes in animals given crude extracts of the pituitary. That would fit with the results in our laboratory. The crude extracts prepared by Dr. Selye contain little or no ACTH activity. Furthermore, we can inhibit the development of pathological changes which are produced by DCA by giving the animals a combination of DCA and ACTH, and we can also inhibit the development of the lesions by giving the animals a combination of DCA and cortisone. These results fit a point of view that we have had for quite a few years. There is an antagonism between the desoxy-like steroids and the 11-oxygenated steroids. We have a number of evidences which fit together in regard to this concept of antagonism. For instance, DCA will induce in experimental rats hypersensitivity to insulin; this hypersensitivity can be counteracted with cortical extracts. DCA produces a hypernatremia. We can restore the concentration of sodium to normal by giving the DCA-treated animals cortisone. Another index of action is brain excitability as measured by electroshock threshold (the amount of current required to induce a seizure in an animal). DCA reduces brain excitability and cortisone increases brain excitability. The two steroids antagonize one another when given together. Part of the action of DCA is to inhibit pituitary ACTH activity. Desoxycorticosterone also antagonizes cortisone-like compounds out in the peripheral tissues, possibly as a result of a competitive inhibition.

A. White: Dr. Sayers, has your experience with the 11-desoxy compounds been limited to DCA?

G. Sayers: We have carried out some experiments with pregnenolone in our brain excitability tests. Pregnenolone and 21-acetoxypregnenolone appear to be inert as far as this particular index is concerned. Even very large doses of these compounds have no influence upon brain excitability—either to reduce brain excitability as is the case of desoxycorticosterone or to increase it as in the case of the cortisone-like compounds.

H. Selye: As regards the antagonism between DCA and cortisone or mineralo-corticoid and gluco-corticoid compounds in general, I think Dr. Sayers' observations are quite in agreement with what we have discussed here in the course of the lecture. I think we might perhaps add to it the point which Dr. Conn raised that this antagonism is not a complete one. One should not consider it in the sense in which, for instance, an acid neutralizes an alkali. Or, if it neutralizes in any one respect, it automatically neutralizes in every other respect. That is not so between mineralo-corticoid and gluco-corticoid compounds. Many of the effects of the two

types of steroids are synergistic. For instance, adrenal atrophy is produced by both mineralo-corticoid and gluco-corticoid compounds. The life of an adrenalectomized animal is maintained by both, and if you give both together, you obtain an even better life maintenance. One particularly important exception to this antagonism rule is the nephrotoxic action of gluco-corticoid and mineralo-corticoid compounds; these compounds are synergistic also in this activity.

As regards the effect of DCA and insulin on sugar metabolism, we could add that in the early stages of our work on the alarm-reaction, we found that the hyperglycemia produced by various stresses (such as traumatic shock, exposure to sudden cold, pain) can be inhibited, and often even reversed by previous DCA treatment. We attributed this to the compensatory atrophy of the adrenal and the consequent inhibition of gluco-corticoid production.

As regards the brain excitability studies, which are of the greatest importance, I wonder whether Dr. Sayers has made any effort to correlate his findings with the anesthetic effect of steroids.

G. Sayers: In our laboratory we haven't been impressed by a correlation between anesthetic effects and brain excitability. However, we would like to screen many more compounds before reaching a definite conclusion.

I would like to make one more point regarding the development of pathologic changes in experimental animals with desoxycorticosterone acetate. We find that a most important aspect of the experimental technique is the fact that it is very difficult to reproduce these lesions. We have attempted to control every possible variable and yet we find that one group of animals will have severe pathologic changes and another, presumably treated in exactly the same way, with no lesions. We have come to the conclusion that desoxycorticosterone acetate is a sensitizing factor, and the real etiological factor is something that we don't know about. Given the right conditions, and in the presence of desoxycorticosterone acetate, factor X will induce lesions. I think this is rather important in view of the discrepancy that we have in regard to the effect of cortisone on the adrenal function and adrenal histology. We have noticed, in contrast to Dr. Selye, that cortisone and ACTH counteract the damage which desoxycorticosterone acetate induces in the kidney.

R. Dorfman: I would like to complicate the picture a little more by relating some experiences with mixtures of 11-desoxycorticosterone and corticosterone. The test animal was the fasting adrenalectomized mouse and the end point liver glycogen. Under the conditions of the experiment 11-desoxycorticosterone at a 2 mg. dose level produces no significant liver glycogen deposition, while corticosterone alone produces a small but significant effect at 100 mg. When the two steroids are administered simultaneously, the liver glycogen deposition is greatly increased over that seen when corticosterone alone is administered. This looks like a true potentiation.

H. Selye: In closing this discussion, I would like to call your attention to one point which seems to me especially important in relation to these STH effects.

It is a little difficult for us, at this point, to think of STH—a hormone which is supposed to stimulate normal growth in length—as a substance in any way involved in the so-called "collagen diseases" and in inflammatory phenomena. However, a few years ago it would have been equally difficult to think of ACTH and cortisone in this sense. In 1941-1943, when the first experiments were done with LAP, our anterior pituitary extract, and DCA, it was noted that these hormones produced hypertensive and rheumatic-like changes in animals. At that time the theory, proposed by our group, that the anterior pituitary and the adrenal cortex may participate in the pathogenesis of rheumatic, allergic, and hypertensive diseases was considered extremely farfetched. It remained essentially in this same position until the workers of the Mayo Clinic made their important contribution by showing the therapeutic value of hormones of the pituitary and adrenal cortex. Thus they taught us how to use hormones for the treatment of these diseases, and treatment is, of course, the most important final aim of medical research.

If STH is to enter into the pathogenesis of the not primarily endocrine diseases, where would it fit in best? Obviously it tends to mimic the effect of mineralo-corticoid compounds; it tends to produce collagen disease-like changes in animals; it might be involved in some way in hypertensive vascular disease, in nephrosclerosis, and in rheumatic-allergic changes. However, the most important thing in this connection is that STH does not give merely an exact reproduction of what we get with mineralo-corticoid compounds. Every change that I have so far been able to produce with DCA, I have been able to reproduce with STH, but the reverse is certainly not true. STH, among other things, has a rather important effect on growth, which DCA does not have.

Second, STH has a marked effect on lymphatic and hematopoietic tissues. The thymus and spleen of animals treated with STH are large and this is accompanied by marked hematologic changes. In our heavy overdosage experiments we even noted an acute splenomegaly, sometimes with splenitis.

The livers of STH overdosed animals were unusually enlarged. At the same time there is proteinuria and hyalin cast formation. This syndrome of the large spleen, the large liver, and the tendency toward proteinuria, is a rather nonspecific one in internal medicine. Many of the general infectious syndromes, "the fevers," the septicemias, cause these manifestations. They are connected with inflammation and, therefore, with connective tissue activation for defensive purposes.

3

Effect of Pregnenolone and Progesterone Upon Contractility, Excitability, and Rhythmicity of Cardiac Muscle

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Introduction

The effects of many steroids on the heart are just beginning to be reported in the literature. Thus Gowdey, Loynes and Waud[1] investigated the effects of a large number of such substances on isolated frog and rabbit hearts. Among others, pregnenolone was found to augment the contractility but showed no effect upon heart rate. Progesterone, however, caused definite reduction in cardiac contractility. Similar observations on the action of progesterone on heart muscle were made by Bernard Rubin,[2] working with the isolated papillary muscle of the cat. Such effects had already been described by early workers who did not have the benefit of purified crystalline materials, but who tested the action of extracts of ovary[3] and corpus luteum[4] upon cardiac contractility.

Both pregnenolone and progesterone are becoming increasingly important therapeutic agents, the former because of some promise it has shown in the treatment of rheumatoid arthritis,[5, 6] and the latter because of its great concentration in the blood during pregnancy and also because of its growing use in the treatment of certain gynecological conditions. In the case of pregnenolone its use in the treatment of certain forms of arthritis necessitates its administration to middle-aged and older patients of whom a fairly substantial number may be expected to suffer also from hypertensive and arteriosclerotic heart disease. In addition there is always the forgotten group of gouty arthritis cases that may be treated with the same agents as patients with rheumatoid arthritis. In the gouty group especially arteriosclerosis and arteriosclerotic heart disease are frequent. It seems important, therefore, to study the effects of both pregnenolone and progesterone on contractility, rhythmicity, and excitability of cardiac muscle not merely in isolated preparations such as the papillary muscle of the cat, but also in the intact animal.

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Method

1. Papillary Muscle Preparation of the Cat.[7, 8] Six male cats (3 to 3.5 kg.) were lightly anesthetized with ether, the chest quickly opened, and as much blood as possible (55–65 cc.) aspirated from the left ventricle and inferior vena cava to furnish homologous serum. The papillary muscles were removed from the right ventricle and the longest and thinnest used for the experimental procedure. Ties were placed around the chorda tendinae and the base of the muscle cut from the ventricular wall.

FIG. 1. Depressant effect of serum containing pregnenolone (100 μg. %) in propylene glycol upon contractility of the isolated cat papillary muscle, compared with the effect of serum and propylene glycol alone in the same experiment. Contractility is expressed as % increase over amplitude in Krebs-Henseleit solution. Note the latent period of 15 minutes before the depressant effect of pregnenolone appears.

One end of the muscle was then connected to a watch spring lever upon which a mirror had been placed, thus enabling photographic recordings of the contractions. The preparation was placed in a glass chamber containing Krebs-Henseleit[9] solution through which a constant stream of 95% oxygen and 5% carbon dioxide was passed. The temperature was maintained at 37.8° C. by means of a water bath. The muscle was driven at a rate of 50/m. by a square wave of 1 and 1.5 msec. duration through platinum electrodes in a lucite holder to which the distal end of the

muscle was attached. The threshold current strength was then determined. The muscle was permitted to reach equilibrium in Krebs-Henseleit solution (this process took at least 1 hour) which was then replaced by 80% homologous serum containing pregnenolone (100 μg./100 cc. serum) dissolved in propylene glycol, or 80% serum containing an equivalent amount of propylene glycol without pregnenolone. The behavior of the

FIG. 2. Effect of serum and pregnenolone (100 μg. %) in propylene glycol upon isolated papillary muscle of cat. The cessation of contractility after remaining in this bath for 30 minutes is demonstrated, as well as the persistence of this effect in Krebs-Henseleit solution and return of contractility when placed in serum containing propylene glycol but not pregnenolone.

preparation was observed for at least 1 hour in each solution and recordings of contractility and threshold values were made every 15–30 minutes.

It has been shown that superthreshold stimuli can in themselves alter excitability.[10] Previous studies on papillary muscle preparations have involved the use of superthreshold stimuli to overcome spontaneous rhythmicity. However, because of these effects and the importance of threshold determinations and rhythmicity changes in this study, threshold stimuli were employed throughout.

2. Excitability Changes in the Intact Dog Heart. Changes in the

excitability cycle of the intact dog heart were studied in 6 male animals (5 kg.) by the method described by Nahum et al.[10] In 5 animals excitability cycles were determined at intervals of 0.5 to 2 hours following intravenous injection of 1 cc. of propylene glycol alone. Three dogs were studied following intravenous administration of pregnenolone in amounts calculated to give an estimated blood level of 100 μg./100 cc. serum dis-

FIG. 3. Effect of pregnenolone upon threshold of excitability of isolated cat papillary muscle. Ordinate represents threshold in volts and abscissa the time during which the muscle was in the various baths. Note the immediate fall in threshold when serum containing pregnenolone is substituted. Curve I represents thresholds at a pulse duration of 1.0 milliseconds and Curve II those at 1.5 milliseconds.

solved in 1 cc. of propylene glycol. Similar studies were made on 3 dogs after injection of progesterone dissolved in propylene glycol in amounts calculated to produce an estimated blood level of 400 μg./100 cc.[13] In each experiment the normal excitability was first determined, followed by a study of the changes produced by propylene glycol. Finally, the effects of the steroid dissolved in propylene glycol were observed. In each experiment simultaneous recording of standard limb leads and unipolar

limb and precordial leads were taken with a Sanborn Tri-Beam Cardiette, at frequent intervals.

Results

Effects of Pregnenolone on Contractility of Isolated Cat Papillary Muscle. In all experiments the results were similar. When serum was substituted for Krebs-Henseleit solution there was an immediate increase in contractility, and in the presence of propylene glycol alone this increase continued for the duration of the observation, the amplitude always

FIG. 4. Effect of propylene glycol on the excitability cycle of the intact dog heart. Ordinate represents the threshold values in milliamperes and abscissa time in milliseconds after the end of S in the unipolar left precordial electrocardiogram. Curve I represents the normal excitability cycle, and Curve II the cycle one hour after intravenous administration of 1 cc. of propylene glycol. P^1 and P^2 represent the beginning of the P wave for Curves I and II respectively. In Curve II note the rise in threshold values, shortening of the threshold phase, and prolongation of the absolute refractory period.

being greater than that in Krebs-Henseleit solution. When the serum contained pregnenolone also, there was a parallel increase in contractility for the first 15 minutes. Following this period, however, a definite depression was observed (Fig. 1). In two experiments the amplitude of con-

traction decreased, within 30 minutes, to the same level as that observed in Krebs-Henseleit solution, and in two others to a value less than 5% above the Krebs-Henseleit baseline. In one experiment contractions ceased completely 30 minutes after the muscle was placed in serum containing pregnenolone and remained absent for 30 minutes more in this solution. The muscle did not regain contractility when bathed for 15 minutes in Krebs-Henseleit solution, but when this was replaced by serum and propylene glycol, contractions immediately returned, and within 15 minutes their amplitude exceeded that originally observed in Krebs-Henseleit solution (Fig. 2).

FIG. 5. Effect of pregnenolone dissolved in propylene glycol upon the excitability cycle of the intact dog heart. Curve I is the control cycle and the curve determined 1½ hours after intravenous injection of propylene glycol. Curve II was determined 40 minutes following injection of pregnenolone (100 μg. %) in propylene glycol (1 cc.), and Curve III 1 hour after Curve II.

Papillary muscle preparations exhibit a normal decay curve. In order to preclude the possibility that the negative inotropic effect observed with pregnenolone was, in reality, a manifestation of this decay, since in the earlier experiments the pregnenolone observations were made last, the order was reversed in two experiments so that the effects of pregnenolone were observed before those of propylene glycol. Whether the pregnenolone was tested first or last, the reduction in contractility occurred only in its presence and may properly be attributed to its action.

Effects of Pregnenolone on Threshold of Excitability of Isolated Papillary Muscle of the Cat. In all papillary muscle experiments preg-

FIG. 6. A. Control electrocardiograms. B. Tracing taken 1½ hours after intravenous injection of propylene glycol. C. Tracing taken 1½ hours after intravenous injection of pregnenolone in propylene glycol. VCR, VCS, and VCL represent unipolar leads over the right apex, mid-sternum, and left apex respectively.

nenolone caused a lowering of threshold. Compared with the threshold in Krebs-Henseleit solution, it was found to fall when the muscle was bathed in serum and propylene glycol, and then declined further when pregnenolone was added (Fig. 3).

Effects of Pregnenolone on the Excitability Cycle of the Intact Dog Heart. Since propylene glycol was the vehicle for the intravenous injection of pregnenolone, the effects of this substance alone on the excitability cycle of the intact dog heart were first established. Propylene glycol invariably slowed the heart rate, and this slowing persisted for several hours. Despite the reduction in rate, which in itself has been shown to reduce threshold,[10] propylene glycol served to raise the threshold so that this may properly be considered a specific effect. In Fig. 4 the influence of propylene glycol upon the excitability cycle is seen. Curve I represents the normal control and Curve II was determined 1 hour after the intravenous administration of 1 cc. of propylene glycol. The threshold for the various phases of excitability has risen and in the threshold period has changed from 0.87 to 1.15 milliamperes, the absolute refractory period is prolonged by 50 msec., and the onset of the threshold period is delayed by 40 msec. These effects

FIG. 7. Effect of progesterone (7 mg.) in propylene glycol (1 cc.) upon the excitability cycle of the intact dog heart. Curve I is the control and Curve II the excitability cycle 40 minutes after the intravenous injection of the progesterone solution. Note the lowering of threshold values.

gradually disappeared after 0.5–2 hours. When the influence of pregnenolone dissolved in propylene glycol was determined in the same animal 2 hours after its injection and the stimulating electrode remaining in the same position, the changes in excitability were no different from those observed with the solvent alone. In Fig. 5, Curve I, determined 1.5 hours after the administration of propylene glycol, is identical with a previously obtained control. Curve II, determined 40 minutes after administration of the same amount of propylene glycol containing pregnenolone, shows

elevation of threshold (1.15–2.65 milliamperes), prolongation of the absolute refractory period (15 msec.) and delay of the onset of the threshold period (25 msec.). These effects are characteristic of the action of propylene glycol. Curve III, determined 1 hour later, is identical with Curve I and the previously obtained control.

FIG. 8. Unipolar limb leads (VF, VR, VL) and standard limb leads (1 and 3) showing configuration of spontaneous extrasystoles following intravenous administration of progesterone in propylene glycol. Each lead shows a normal beat followed by an extrasystole.

Effects of Pregnenolone on Rhythmicity. In two of the isolated papillary muscle experiments pregnenolone caused prompt development of spontaneous rhythm, which persisted as long as this substance was present in the serum.

In each intact dog experiment a striking influence of pregnenolone on cardiac muscle was the production of ventricular extrasystoles. The

spontaneous ectopic beats were identical in configuration with the forced beats and therefore originated at the site of the electrode.

Effects of Pregnenolone on the Electrocardiogram. In serial electrocardiograms taken at intervals during each experiment the only abnormality that could be attributed to pregnenolone was the appearance of spontaneous ectopic beats (Fig. 6).

Effects of Progesterone on the Excitability Cycle of the Intact Dog Heart. Fig. 7 illustrates the effect of progesterone dissolved in propylene glycol on the normal dog excitability cycle. Curve I represents the normal control, while Curve II was determined 40 minutes after the intravenous injection of progesterone. As can be seen, the threshold during the various phases of excitability has decreased as compared with that of the control. The absolute refractory period has been prolonged, and the onset of the

TABLE I

PROPERTY	PREPARATION	PREGNENOLONE	PROGESTERONE
CONTRACTILITY	PAPILLARY MUSCLE	DECREASED	DECREASED
EXCITABILITY	PAPILLARY MUSCLE	INCREASED	
	INTACT ANIMAL	UNCHANGED	INCREASED
RHYTHMICITY	PAPILLARY MUSCLE	INCREASED	
	INTACT ANIMAL	INCREASED	INCREASED

threshold period has been delayed. A comparison of these curves with those following the injection of propylene glycol shows that the change in absolute refractory period is quite similar to that produced by this substance and may very well be a propylene glycol effect.

Effects of Progesterone on Rhythmicity of the Intact Dog Heart. Ventricular extrasystoles arising from the site of the stimulating electrode were observed in each experiment after the administration of progesterone, and this effect is comparable to that of pregnenolone. These appeared within 30 minutes after injection and persisted for the duration of the experiment (3–4 hrs.).

Effects of Progesterone on the Electrocardiogram. Studies of the effects of progesterone on the electrocardiogram are in progress. Ventricular extrasystoles were observed in serial tracings taken at frequent intervals after the injection of progesterone (Fig. 8).

A general qualitative summary of the results of this study is presented in Table I.

Discussion

These experiments demonstrate some striking effects of pregnenolone on the fundamental properties of cardiac muscle. In the isolated papillary muscle of the cat, contractility was depressed when the muscle was bathed in pregnenolone dissolved in propylene glycol and added to serum to obtain a concentration of 100 μg. per cent. This depression was not observed when the serum contained an equivalent amount of propylene glycol alone, and may properly be attributed to the pregnenolone. It is interesting to note that the effect did not appear until 15 minutes after the preparation had been bathed in serum containing pregnenolone. This interval may represent the time required for building up a concentration within the myocardial cells sufficient to produce the effect. In this connection, Davis and co-workers[11] examined the uptake of radioactive sulfur by various tissues of the rat after intravenous injection of labeled estrone sulfate, and found that a measurable, though small, amount was present in the heart after 15 minutes. This time lag for the development of inotropic change is in contrast to the actions of pregnenolone on both excitability and rhythmicity, which appear promptly.

When pregnenolone dissolved in propylene glycol was administered intravenously to an anesthetized dog, no definite changes in the threshold of excitability which could be attributed to pregnenolone were uncovered. It may be that the dosage employed was insufficient to reveal the changes either because the blood level declined too rapidly or because the metabolism of the living heart quickly disposed of the intracellular concentration. The 100 μg. per cent concentration was chosen for several reasons. First, since the absorption of pregnenolone is slow and uncertain from both the intestinal tract and muscle, 100 μg. per cent would in all probability represent a high concentration in any patient receiving pregnenolone therapeutically. Second, because of the limited solubility of pregnenolone in propylene glycol, it was possible to administer only an amount sufficient to produce the aforementioned concentration in 1 cc. of solvent. To achieve higher concentrations a large amount of propylene glycol would be necessary and, as can be seen from Fig. 4, 1 cc. raised the threshold of excitability significantly and a greater volume would have masked any lowering of threshold that might have resulted from the pregnenolone itself.

Although excitability changes in the intact dog heart were not observed, the increase in rhythmicity found when the papillary muscle of the cat was exposed to pregnenolone in homologous serum was likewise observed in the intact animal. In each instance, after injection of pregnenolone intravenously, spontaneous ventricular extrasystoles appeared and persisted. It must not be assumed that because the spontaneous ectopic beats originated from the site of the stimulating electrode that they were due entirely to its presence. They occurred only infrequently under normal circumstances and after injection of propylene glycol. Since

spontaneous rhythm developed in the isolated papillary muscle as well, it may be concluded that increase in rhythmicity is a specific effect of pregnenolone on heart muscle.

The action of progesterone upon the papillary muscle of the cat has already been described by Rubin,[2] who showed that this substance (like pregnenolone) produced a definite decrease in contractility after 15 minutes of exposure. This negative inotropic effect of progesterone increased with serum concentration, being slight at 100 μg. per cent and considerable at 400 μg. per cent. Since these results were in agreement with all former workers, the study was not repeated here. However, the effects of progesterone on threshold of excitability and rhythmicity had not been studied, and therefore the experiments upon the intact dog heart reported here were undertaken. It should be noted that the concentrations of progesterone employed were considerably higher than those of pregnenolone, for two reasons. In the first place, progesterone is much more soluble in propylene glycol and therefore larger amounts dissolved in a small volume of solvent could be injected. In the second place, the disappearance of progesterone from the blood is known to be rapid,[12] and, if it were comparable to the rate of disappearance of estrone, as described by Hertz et al.,[13] then 7,000 μg. would have to be injected in order to produce a 400 μg. per cent concentration 2–4 hours later when the propylene glycol effects had disappeared and the influence of progesterone established. It is significant that the threshold of excitability dropped in each instance in spite of the tendency to elevation of the threshold that would occur from the action of propylene glycol alone. It may therefore properly be accepted that progesterone reduces the threshold of excitability in the intact dog heart. It may be interesting here to call attention to the similarity between progesterone and digitalis in this regard, for it has been shown that digitalis also lowers the threshold of excitability in the intact dog heart.[14] The increase in rhythmicity that occurred in each experiment after the injection of progesterone is altogether comparable to that described for the action of pregnenolone.

It should be recalled that during some phases of pregnancy progesterone levels as high as or higher than those experimented with here are present. Nevertheless, ectopic beats are not frequent. However, when such patients receive digitalis, which also lowers threshold, or suffer from intercurrent infection which may increase cardiac irritability, then the genesis of ectopic beats or rhythms in these patients could receive adequate explanation. This eventuality may not be so important in those patients receiving therapeutic doses of pregnenolone since the blood level could hardly be expected to rise high enough to produce significant changes in cardiac activity. However, should methods of intravenous administration be developed, the effects of this steroid upon cardiac contractility, excitability, and rhythmicity would have to be taken seriously into account, especially since many patients to whom it would be administered already suffer from some form of cardiac degenerative disease.

Summary and Conclusions

The effect of pregnenolone upon heart muscle was studied in both the isolated papillary muscle of the cat and the intact dog heart. It was found that pregnenolone in concentration of 100 μg. per cent depressed contractility and lowered threshold in isolated preparations, and increased rhythmicity in the intact dog heart.

The action of progesterone on the intact dog heart was to lower the threshold of excitability and to produce spontaneous extrasystoles.

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Discussion

R. Mendez: There are certain things which I may mention that result from differences in appreciation of technique or differences in appreciation of nomenclature. I think that Dr. Nahum and myself will agree on these. I myself am not very fond of the papillary muscle of the cat. Also, emphasis should be given to the fact that the actions mentioned are on the papillary muscle of the cat and not on the mammalian heart. There are ways of measuring the contractility of the heart, not only in the normal heart but in the hyperdynamic heart. Dr. Nahum, of course, knows these well. I am under the impression that the papillary muscle of the cat is a dangerous tool.

In regard to the excitability of the heart I'm very pleased that Dr. Nahum has taken the point of view mentioned in his talk because he knows as well as I do that there is a lot of confusion in textbooks about the definition of excitability, and that there are many good books of pharmacology which confound the excitability with the spontaneity of the heart. Dr. Nahum has properly measured the excitability cycle.

Dr. Nahum has talked about rhythmicity and this is where nomenclature differences arise. He spoke also of spontaneous discharges. I think that spontaneous discharges are much better than rhythmicity. Coming back to the excitability, I think that discussion of the excitability of a particular tissue in the heart should not be confused with excitability of the whole heart. Dr. Nahum has been talking about the excitability of the heart due to digitalis. I have had experience with this point since I have a paper now on the excitability of the heart under digitalis administration. My experiments show that the excitability of the heart decreases after the first dose of drug, but the excitability of the ventricle may increase; these effects are separated with different doses. There is a great difference between the excitability of the various portions of the heart.

Also, Dr. Nahum, you have been talking about changes in rhythmicity in the therapeutic range of the drug. Have you ever found changes in the rhythmicity of the heart with therapeutic doses of digitalis or any of the digitalis compounds?

Finally, Dr. Nahum says he is under the impression that pregnenolone would alter or add to the digitalis effect. Whether there is an effect or not, I don't want to have people who are not heart specialists under the impression that they must be afraid of using this substance when there is no good experimental evidence to prove pregnenolone has an effect on the human subject's heart.

L. H. Nahum: I think Dr. Mendez has raised a worthwhile point, i.e. what importance to attribute to the papillary muscle of the cat as an experimental technique. Now, it is perfectly true, as I pointed out in these

slides, that there is a decay curve in the papillary muscle because the inside of the muscle is not oxygenated, and is progressively dying, and that the contractility progressively declines. However, it declines very slowly so that if you interpose an experimental procedure upon it, you can see at once the difference between the normal decay curve and the result of the experimental procedure; this is really the way everybody has looked at the isolated papillary muscle of the cat as a test substance. It's only adequate if you take into account the slow decline of contractility normally occurring. Rapid changes in contractility which result from the experiment are clearly defined and, therefore, can and have properly been considered as due to the experimental procedure. In fact, most of the experiments which now form the foundation of our knowledge of the effect of glycosides were performed on just this type of preparation, and the results which are now used in every textbook of pharmacology are an enduring monument to this preparation. Furthermore, I should draw attention to the fact that in this presentation, excitability and automaticity were found to vary similarly in the papillary muscle and the intact dog heart.

Dr. Mendez also raised another point which, I assure him, we took into account, and that is the recognition that different portions of the heart do have different excitability cycles. For example, with our methods, we established the excitability cycle at the apex of the heart and also the excitability cycle at the base of the heart, and it is perfectly true that the excitability varies. The apex normally has a lower threshold, while at the base the threshold is normally higher. We weren't testing the excitability of the heart as a whole—no one can. We were merely testing the excitability of one area, but if this area shows a change in excitability—that is, if this area has a certain threshold now, and after the experiment has a higher or lower threshold—I think it's valid to conclude that the excitability has changed as a result of the experimental procedure, and that all regions of the ventricle vary similarly.

Another point Dr. Mendez emphasized is the difference between excitability and rhythmicity. The way I like to think of it is this: The threshold of the membrane, which we establish by determining the minimal current strength which will cause a discharge, is the excitability threshold. This doesn't imply that spontaneous rhythms will necessarily result from changes in the threshold. There must be added to it another factor to explain spontaneous rhythm and that is the potential changes inside the cell, which we sometimes call the inner stimulus. It's the interaction between the inner stimulus and the level of excitability of the membrane which finally causes spontaneous discharge. If the threshold falls, it's possible to obtain a spontaneous discharge on that basis alone if the inner stimulus remains at the original potential. If the threshold rises, it is still possible to observe spontaneous discharge if the inner stimulus, that is, the potential built up inside the cell, rises sufficiently to exceed the threshold of the membrane. It is the interaction between the inner stimulus and the level of excitability which finally determines

whether or not you are going to have a spontaneous discharge.

Another point raised is the question of whether digitalis can produce ectopic rhythms. Dr. Mendez' remarks on this point are completely at variance with established experience. Everyone who has ever employed digitalis should know that the development of ectopic rhythms in treating heart disease as a result of digitalis administration is an important danger signal. We have given fairly large doses of digitalis to normal animals experimentally and have frequently observed, as has practically every other investigator, that ectopic rhythms occur readily, more so with some digitalis glycosides than with others.

There is no question at all that digitalis may produce, and often does produce, ectopic rhythms in heart disease, and even in the normal heart, though in this case it may require a larger dose.

The last question was whether we can tell whether there is going to be an additive effect between digitalis and the steroids here tested? You can't tell in advance. The only point I made was that inasmuch as they do have parallel actions the possibility of an additive effect exists and has to be taken into account.

A. White: Has anyone else had clinical experience with any of the steroids under discussion with respect to possible cardiac action?

R. Hertz: Patients with cervical carcinoma who were given massive doses of progesterone in the form of 250 mg. daily up to 60 to 90 days exhibited no clinical manifestations of cardiac defect. Practically all of these patients underwent extensive surgery following this therapy and their cardiac reactions during anesthesia and their postoperative course were perfectly normal.

L. Greenman: We have observed no alteration in cardiac rhythm in a small number of patients receiving 5 gm. of Δ^5-pregnenolone per day by mouth for five days in divided doses and in patients receiving 0.5 gm. to 1 gm. of the substance per day intramuscularly in divided doses for periods up to 21 days. Dr. Danowski will present some of these patients in more detail later.

L. H. Nahum: Dr. Greenman, did you by any chance make estimations of the blood levels in these patients? This information may give some sort of a comparison between your results and the study I have just reported.

L. Greenman: We did not measure blood levels since we have no adequate methods for so doing. It may be helpful to relate patient size to dosage in order to estimate grossly the levels. The patients to whom we have given these amounts of pregnenolone weighed 15 to 25 kg. If we assume the hormone is distributed in the extracellular fluid, the concentrations would be higher than 100 μg. per cent; if distributed throughout total

body water the values would approximate this figure. I would like to emphasize that this is entirely assumption without actual measurement or knowledge of absorption, utilization, and excretion. In addition, we encountered local reactions when we administered pregnenolone acetate parenterally.

L. H. Nahum: I suppose you took into account the rate of absorption in your estimation of what the blood levels might be?

L. Greenman: I cannot offer a valid estimate of the blood levels.

K. Dobriner: I would like to know for how many days you gave these large amounts of pregnenolone. The reason for this question is the insolubility of pregnenolone. If you want measurable levels of this steroid it would probably have to be given in large amounts for a number of days.

L. Greenman: We employed several dosages and our patients varied in size. I shall cite some of the extreme examples: A 75 kg. adult received 7 gm. of 21-acetoxypregnenolone intramuscularly in divided doses each day for 10 days. A 3½-year-old girl weighing about 15 kg. ingested 5 gm. of Δ^5-pregnenolone in divided doses for 5 days. Finally, a 20 kg. boy, 12 years old, received 0.5 gm. of Δ^5-pregnenolone intramuscularly per day, again in divided amounts, for approximately 3 weeks, and another child of similar size and age was given 1 gm. a day intramuscularly of 21-acetoxypregnenolone for 5 weeks.

4

Studies of Steroids for Inhibition of Normal and Abnormal Growth in Experimental Animals*

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The program discussed in this paper was initiated to assist in the examination of steroids for biologic activity—especially for antitumor effects. The lack of complete satisfaction with procedures to study steroids for antiarthritic activity has suggested that the biologic activities to be described might be of interest to this symposium. It is not claimed, however, that the data presented herein on the inhibiting effects of steroids on normal growth and on certain tumors in mice have an important significance for rheumatoid arthritis or possibly for more than one type of malignancy. Preliminary tumor-inhibition data from studies in animals indicate their usefulness in developing leads for human studies. In particular there will be described a screening study against mouse lymphosarcoma, which appears to be a good procedure for selecting those worthy of clinical trial from the numerous steroids now becoming available.

This report includes studies from the Division of Experimental Chemotherapy and portions of several other biologic and biochemical investigations of steroids being conducted in other departments of the Sloan-Kettering Institute. Dr. David A. Karnofsky has screened steroids for their ability to inhibit the development of the chick embryo. Dr. Kanematsu Sugiura has studied steroids for their ability to inhibit the growth of a variety of transplantable tumors in mice and rats. In addition, Dr. George W. Woolley, Division of Steroid Biology, has permitted us to refer to his studies of a few steroids for their influence on blood, organs, and tumors in lymphosarcoma-bearing mice. Dr. William Money in the Division of Clinical Endocrinology has provided the data on the effect of steroids on the organs of normal rats, and Dr. Oscar

* We wish to acknowledge the extensive assistance of Dr. Konrad Dobriner in this program.

We also wish to acknowledge support for the several aspects of this program from the American Cancer Society and the National Cancer Institute of the United States Public Health Service.

Bodansky in the Division of Clinical Biochemistry has furnished data, in press, on the influence of Compound L upon vitamin A levels. Although the 11-desoxy steroids are primarily the subject matter of this symposium, our results with the 11-oxygenated steroids will be reported as a background for the data on the 11-desoxy steroids.

The classic experimental and clinical studies of the influence of the sex hormones on tumors by Lacassagne,[1] Allen,[2] Lipschutz,[3] Gardner,[4] Huggins,[5] Nathanson,[6] Adair and co-workers,[7, 8] and Haddow and his associates[9] are well known. Our study of steroids in a spectrum of animal tumors,[10] stimulated by the discovery of the usefulness of cortisone in rheumatoid arthritis,[11] has not yet been developed to include tumors responding primarily to androgenic, progestational, or estrogenic hormones. We have concentrated upon a search for compounds which might have a cortisone-like action against experimental tumors. Our results with cortisone were foreshadowed by those of Heilman and Kendall[12] on the inhibition of mouse lymphosarcoma and by Murphy and Sturm[13] on the inhibition of development of transmitted leukemia in rats by adrenal cortical extracts. Clinical studies with cortisone and ACTH were under way in patients by Pearson et al.[14] at the time our experiments with cortisone in experimental animals were initiated. The adverse effect of cortisone on tumors in animals has been extended by others (Higgins et al.,[15] Emerson et al.,[16] Woolley,[17] and Baker and Whitaker[18]).

In our exploratory studies we were interested in determining:

1. Whether the lymphosarcomas in our chemotherapy program would respond to cortisone under the test conditions employed.

2. Whether types of tumors other than those of lymphoid origin would be affected.

3. Whether there are differences in patterns of activity of different steroids on different tumors.

Early in the program it was realized that some steroids would be available in amounts too limited even for preliminary trials in the animals. As a consequence tests were initiated in the chick embryo and, after cortisone showed a marked activity against the embryo, all steroids have been screened with the developing chick embryo.[19] The effects from cortisone were so striking that it appeared possible to detect easily limited amounts of cortisone-like activity either from small amounts of material or from larger amounts of less active substances. The relationship of this gross effect on the embryo to recognized types of steroid activity must be determined by further studies.

Landauer[20] first reported that an extract of adrenal cortex would inhibit the growth of chick embryos. The marked inhibitory action of cortisone acetate observed by Karnofsky et al.[19, 21, 22] is shown in Fig. 1. In the earlier experiments saline suspensions of 2 to 4 mg. of steroid were injected into the yolk sac of the four-day-old embryo. The embryos surviving to 18 days showed a moderate to severe stunting with generalized baldness. The effect was explored in more detail, and a range of growth inhibition was found. Fig. 2 shows this range divided arbitrarily into four

FIG. 1. Inhibition of chick embryo development by cortisone. Left chick, normal 18-day-old embryo. Other two chick embryos of same age show maximum stunting from cortisone.

FIG. 2. Gradations in effects from cortisone.

stages or degrees of effect. The embryos dying before the eighteenth day are small, pale, and edematous and have unusually large eyes in relation to body size. Often they are eviscerated. Those surviving to 18 days show a characteristic syndrome. The embryo is small; the yolk sac and chorio-allantoic membrane have not formed completely; there is consequently free yolk and albumin present which is not enveloped by the membranes. The embryos adopt a characteristic curved position, and the amnion is

TABLE I

AVERAGE RESULTS OF EFFECTS ON CHICK EMBRYO FROM VARIOUS AMOUNTS OF CORTISONE ADMINISTERED BY SEVERAL ROUTES*

Dose Mg./Egg	4-Day Yolk Sac Embryo Effect (1-4+, Average)	4-Day Yolk Sac Weight, Gm. (Average)	8-Day Yolk Sac Embryo Effect (1-4+, Average)	8-Day Yolk Sac Weight, Gm. (Average)	8-Day Chorioallantois Embryo Effect (1-4+, Average)	8-Day Chorioallantois Weight, Gm. (Average)
			(Sacrificed at 17-18 Days)			
0.2					0 (3)	16.9
0.5	+0.8 (5)†	14.5	0 (4)	17.4	+2.7 (7)	10.9
1.0	+1.3 (21)	13.0	+1.0 (4)	13.8	+2.5 (2)	10.5
2.0	+2.4 (19)	10.8	+2.8 (11)	8.8	+4.0 (8)	5.2
4.0	+4.0 (3)	10.1	+4.0 (3)	4.4		

* The effects are graded from 1 to 4+ as presented in Fig. 2.
† Number of embryos.

drawn tightly around them. The embryo is pale and the feathers are practically absent; evisceration is common. Embryos showing intermediate effects survive for 21 days but fail to hatch.

The effect of cortisone on the embryo was determined in relation to the dose for yolk sac injections at four days, and at eight days for the chorioallantoic route of injection. In the latter the maximum inhibition was obtained with 2 mg. of cortisone acetate; larger amounts were required by the other route (Table I). Study of the relationship of the time of

TABLE II

ACTIVITY OF 2 MG./EGG OF CORTISONE ACETATE INJECTED INTO THE YOLK SAC AND ON CHORIOALLANTOIC MEMBRANE (0-15 DAYS OF AGE)*

Time of Injection, Days	Route: Yolk Sac Embryo Effect (1-4+, Average)	Yolk Sac Weight, Gm. (Average)	Chorioallantois Embryo Effect (1-4+, Average)	Chorioallantois Weight, Gm. (Average)
		(Sacrificed at 17-18 Days)		
0	+2.1 (6)‡	10.0	.....	..
2	+2.1 (6)	11.3	.....	..
4	+1.8 (7)	10.7	.....	..
6	+2.1 (5)	10.0	.....	..
8	+2.8 (8)	8.4	+4.0 (6)	5.2
10	+2.2 (9)	10.0	+3.8 (4)	7.4
12	+3.1 (14)	7.1	+2.6 (9)	7.8
15	0 (9)	17.5†	0 (9)	15.8†
Controls	(16 to 21 Gm.)			

* The effects are graded from 1 to 4+ as presented in Fig. 2.
† Sacrificed, 19 days.
‡ Number of embryos.

dosage to the degree of inhibition revealed that the effect could still be obtained when the dosage by either route was delayed as late as the twelfth day (Table II). The physiologic significance of this observation may be clarified by histologic studies still in progress.

The present assay of steroids for cortisone-like activity in the embryo is as follows: The steroid solution or suspension is placed on the chorio-

TABLE III

INHIBITION IN CHICK EMBRYO DEVELOPMENT BY STEROIDS

Name	*Source**	*Minimum Dose for Selected Inhibition† Mg. Steroid/Egg*
Kendall's Compound F acetate	I	0.015
Corticosterone	J	0.15
Kendall's Compound A acetate	E	0.25
21-Desoxycortisone	E,H	0.3
11-Ketoprogesterone	E	0.42
Cortisone acetate	E	0.45
11-Desoxycorticosterone acetate	B,F	0.9
21-Acetoxypregnane-3,20-dione	B	2.0
Progesterone	A,B,G	2.4
Reichstein's Compound S acetate	D,E,I	2.7
3β-Hydroxy-21-acetoxy-Δ^5-pregnene-20-one	B,G	3.0
17α-Hydroxy progesterone	B,I	3.0
17α-Hydroxy-21-acetoxy-Δ^1-allopregnene-3,20-dione	B	8.5
3β-Hydroxy-Δ^5-pregnene-20-one (pregnenolone)	G,B	9.0
3β,21-Diacetoxyallopregnane-20-one	B	9.0

* We wish to acknowledge our indebtedness to the sources of the steroids designated in Tables III, IV, V, and VI by letters as follows: A, Ayerst, McKenna and Harrison (Dr. Gordon Grant); B, Chemical Specialties—Syntex (Dr. I. V. Sollins); C, Ciba Pharmaceutical Products (Dr. C. Scholz); D, Glidden Company (Dr. P. Julian); E, Merck and Company (Dr. M. Tishler, Dr. A. Gibson); F, Roche-Organon; G, Schering Corporation (Dr. E. B. Hershberg, Dr. E. Henderson); H, Sloan-Kettering Institute (Dr. T. F. Gallagher); I, Upjohn Company (Dr. M. Kuizenga, Dr. W. Haines, Dr. W. Hailman); J, Worcester Foundation for Experimental Biology (Dr. G. Pincus).

† The degree of inhibition selected corresponds roughly to that between grade 2 and 3 (Fig. 2) and is believed to be a satisfactory degree of inhibition for comparative purposes.

allantoic membrane of the eight-day-old developing chick embryo. Ten days later the embryo is sacrificed and graded for growth inhibition. With this technique a large number of steroids have been tested. The active ones are presented in Table III. Those showing activity in the preliminary test have been studied further to define more accurately the minimal dose required to produce a selected degree of inhibition chosen for comparative purposes. Table III shows that the 11-oxygenated steroids are most effective, but a number of 11-desoxy compounds have also inhibited the

embryo at higher dosages. Compound F is outstandingly active. It is of interest that there is little difference in the activity of 21-desoxycortisone and cortisone acetate, for, as we will see later, the antitumor activity of cortisone is nearly lost with removal of the 21-hydroxy group. Over 40 other steroids have been tested at 10 or 20 mg. per egg without showing any inhibition of the embryos. They are listed in Table IV with the highest level used. The effects observed with the active steroids differ from those produced by x-rays and any other types of chemicals. The exact nature of the activity is yet to be determined. It is believed that the chick embryo can offer a useful tool for detecting small amounts of biologically active steroids; however, the steroids active against the chick embryo do not necessarily show similarities in patterns of other biologic activities. It will be seen, for example, that the group of steroids active against mouse lymphosarcoma is more restricted than those active against the

TABLE IV

STEROIDS INACTIVE IN INHIBITING CHICK EMBRYO GROWTH

Compound	*Source**	*Maximum Dose Tested Mg./Egg*
I. C 21—Steroids with an 11-oxygen function and saturated A and B rings		
3α-Acetoxypregnane-11,20-dione	E	20
4 (?), 21-Diacetoxy-17α-hydroxypregnane-3,11,20-trione	E	10
17α-Hydroxy-21-acetoxypregnane-3,11,20-trione	E	10
17α-Hydroxy-3α,21-diacetoxypregnane-11,20-dione	E	20
II. C 21—Steroids without an 11-oxygen function and with saturated A and B rings		
3β-Hydroxyallopregnane-20-one	G	20
17α-Hydroxy-3β-acetoxyallopregnane-20-one	B	20
3α,12α-Dihydroxy-21-acetoxypregnane-20-one	C	5
17α-Hydroxy-3β,21-diacetoxyallopregnane-20-one	B	20
17α-Hydroxy-3β-acetoxypregnane-20-one	B	20
17α-Hydroxy-21-acetoxyallopregnane-3,20-dione	B	10
3α,12α,21-Triacetoxypregnane-20-one	B	20
III. C 21—Steroids without an 11-oxygen and with an unsaturation at C 1		
17α-Hydroxy-Δ^{1}-allopregnane-3,20-dione	B	20
17β-Acetyl-$\Delta^{1,3,5}$-estratriene-3,17α-diol	B	20

* Refer to footnote * of Table III.

TABLE IV—*(Continued)*

Compound	*Source**	*Maximum Dose Tested Mg./Egg*
IV. C 21—Steroids without an 11-oxygen function and no carbonyl at C 20		
3-Keto-Δ^4-pregnene-17α,20,21-triol	C	10
3-Keto-Δ^4-pregnene-17β,20,21-triol	G	20
17α-Hydroxy-20β,21-diacetoxy-Δ^4-pregnene-3-one	D,I	20
Δ^5-Pregnene-3β,17 (α and β),20-triol	G	20
17α,20β-Dihydroxy-Δ^4-pregnene-3-one	C	20
Δ^5-Pregnene-3β,17β-diol	C	20
21-Acetoxy-$\Delta^{4,17}$-pregnadiene-3-one	C	20
3β-Acetoxy-Δ^5-bisnorcholenic acid	D	20
$\Delta^{5,17}$-Pregnadiene-3β-ol	C	20
V. C 21—Steroids without an 11-oxygen function and with unsaturation at C-16		
3β-Acetoxy-$\Delta^{5,16}$-pregnadiene-20-one	G	20
$\Delta^{4,16}$-Pregnadiene-3,20-dione	B	20
3β-Acetoxy-16-methyl-$\Delta^{5,16}$-pregnadiene-20-one	B	20
VI. C 21—Steroids, miscellaneous		
3β,21-Diacetoxy-Δ^5-pregnene-20-one	D	20
3β-Acetoxy-17α-hydroxy-Δ^5-pregnene-20-one	D	20
3β-Hydroxy-16β-methyl-Δ^5-pregnene-20-one	B	20
VII. C 19—Steroids		
$\Delta^{1,4}$-Androstadiene-3,17-dione	B	5
2?, 17β-Diacetoxy-Δ^4-androstene-3-one	B	20
Δ^1-Androstene-3,17-dione	B	10
Δ^5-Androstene-3β,17β-diol	B	20
Δ^4-Androstene-3,17-dione	B	10
17α-Acetoxy-Δ^4-androstene-3-one	B	20

* Refer to footnote * of Table III.

chick embryo. The studies of the steroids against the embryo are being extended to include newly hatched (two- to three-day-old) chicks and baby mice. In the chicks, observations have been made on the weight gain after ten days of injections of steroids. Inhibitions have been found thus far as follows: a pronounced effect from Compound F, an effect from corticosterone, smaller activities from cortisone and from DOCA, little inhibition from Compound S, and none from progesterone, pregnenolone, testosterone, dihydro- and tetrahydrocortisone, Compounds A and L, and ACTH. In baby mice, observations made after nine days of injections have shown a retarded weight gain, alteration in hair growth, and an

early eruption of the teeth with Compound F and cortisone at 0.02 mg./day, Compound A at 0.05 mg./day, 21-desoxycortisone at 0.5 mg./day, DOCA at 2.0 mg./day, and ACTH at 3 mg./day.

All steroids available in adequate amounts are being tested for their activity against a variety of mouse and rat tumors. Much of this study has been conducted without consideration of the results in the chick embryo test, although naturally a special attempt has been made to test promptly all steroids active against the chick embryo. The antitumor activity has been determined by a test of the ability of the steroids to inhibit the growth of tumor implants. After the mice have been weighed individually, tumor implants are made subcutaneously into the axillary region. Twenty-four hours later the steroids as saline solutions or suspensions are injected subcutaneously in maximally tolerated doses into groups of five mice each. The injections are continued daily for a week. At the end of the injection period the mice are reweighed and the tumors measured in two diameters by calipers and the measurements repeated at weekly intervals.

The results obtained with cortisone against a variety of tumors are included in Table V. The maximum tolerated dose is 37.5 mg./kg./day (0.75 mg./mouse/day) and it has shown delayed, complicated metabolic side-effects. The strongest inhibitions have been obtained with the lymphosarcomas and osteogenic sarcomas. Mammary adenocarcinoma EO 771 has been inhibited moderately, while Sarcoma 180 has not been significantly affected at maximally tolerated doses. A similar range of activity, although weaker in degree, has been shown by ACTH in doses of 100 mg./kg./day once or twice daily. At the same total dosage given every two hours for a week, there is a wider range of tumor inhibition.

As the mice receiving cortisone showed appreciable weight losses relative to the untreated controls, determinations of the influence of decreased caloric intake have been made to ascertain whether the observed tumor inhibitions may have been due to inanition. Tumor-bearing mice which normally would eat *ad lib.* approximately 3 gm. of Purina Laboratory Chow a day were restricted to 1 gm. a day for nine or ten days. This schedule caused weight losses similar to those seen in the cortisone-treated mice. The decreased caloric intake caused no inhibition or, at most, a slight inhibition (±) of all the tumors except mammary adenocarcinoma EO 771. In at least one experiment this tumor was significantly inhibited by the decreased food intake.

The degrees of inhibition of the osteogenic sarcomas and the lymphosarcomas are shown in Figs. 3 and 4. In the case of the lymphosarcoma, an increase in survival time also has been noted. Histologic studies have not revealed more than a mixture of viable cells and cells with degenerating nuclei in the treated lymphosarcomas.

The results with cortisone encouraged study of numerous steroids in the spectrum of tumors with particular emphasis on the lymphosarcomas. Over 60 steroids have been screened in this way. In Fig. 5 are presented the formulas of a group of closely related steroids. These include a number of 11-oxygenated steroids which have been most active. Compound F

TABLE V

EFFECT OF CORTISONE ON VARIOUS TUMORS IN MICE*

Dose mg./kg./day	*Sarcoma 180*	*Sarcoma T241*	*Sarcoma Ma 387*	*Carcinoma 1025*	*Mammary Adeno-carcinoma EO 771*	*Harding-Passey Melanoma*	*Wagner Osteogenic Sarcoma*	*Ridgway Osteogenic Sarcoma*	*Patterson Lympho-sarcoma*	*Mecca Lympho-sarcoma*
37.5	—	+	—	±	+	—	++	++	++	++
25.0	..	..	..	..	..	..	....	++	++	++
12.5	..	..	..	..	..	..	....	±	+	++

* — indicates no effect; ± indicates slight inhibition; + indicates moderate inhibition; ++ indicates marked inhibition.

FIG. 3. Area diagrams of osteogenic sarcomas in ten control mice and ten mice receiving cortisone (37.5 mg./kg./day) for seven days. Measurements were recorded at weekly intervals after tumor transplantation.

FIG. 4. Area diagrams of lymphosarcomas in ten control mice and ten mice receiving cortisone (37.5 mg./kg./day) for seven days. Measurements were recorded at weekly intervals after tumor transplantation.

appears to be nearly as active as cortisone, whereas three or four times the amount of Compound A is required for comparable activity. Corticosterone has a lower activity; inadequate supplies have prevented an accurate titration of its activity. 21-Desoxycortisone has a quite low degree of activity, possibly one-twentieth to one-fiftieth that of cortisone. Dihydro- and tetrahydrocortisone have shown no activity. The structural aspects of these compounds are listed in Table VI. Table VII presents similar details for a group of steroids which have not inhibited the lymphosarcomas, even

FIG. 5. Formulas of lymphosarcoma inhibitory steroids and closely related steroids.

though most of them have been tested at five to ten times the maximum dose level of cortisone. These doses are at least 15 to 30 times the minimum amount of cortisone used to show an inhibition of the tumors. Although the inactive compounds in Table VII show a number of variations in structure, all of them lack an 11-oxygen function. Thus far, it appears that the most active steroids require an 11-oxygen function and a 3-keto group with a Δ^4-conjugated unsaturation.[23] The 17-hydroxy group is not essential but appears to increase the effectiveness. A 20-keto group has been present in the active steroids, but there has not been an opportunity to test the corresponding 20-hydroxy compounds. The 21-hydroxy group is at least quantitatively very important, if not essential, for the antitumor activity.

Recent studies have indicated in a preliminary way a small number of 11-desoxy steroids which in high doses show a weak inhibition of the lymphosarcoma. These are: 3β,21-diacetoxy-Δ^5-pregnene-20-one, 3β-acetoxy-16-methyl-$\Delta^{5,16}$-pregnadiene-20-one, 3β-hydroxy-16β-methyl-Δ^5-pregnene-20-one, and several others of the pregnadiene and androstadiene

TABLE VI

STRUCTURAL ASPECTS OF EFFECTIVE AND RELATED STEROIDS IN THE INHIBITION OF MOUSE LYMPHOSARCOMA

Compound	*Source**	*Dose MG·/KG·/DAY*	*Activity*†	*11-KETO*	*11-OH*	*Δ^4-3-KETO*	*3-OH*	*20-KETO*	*17-OH*	*21-OH*
Cortisone acetate	E	37.5	+	+	−	+	−	+	+	+
Compound F acetate	I	37.5	+	−	+	+	−	+	+	+
Compound A acetate (17-desoxycortisone acetate)	E	125	+	+	−	+	−	+	−	+
Corticosterone	J	125	±	−	+	+	−	+	−	+
21-Desoxycortisone	E, H	375	− to ±	+	−	+	−	+	+	−
Dihydrocortisone (17α-hydroxy-21-acetoxypregnane-3, 11, 20-trione)	E	375	−	+	−	−	−	+	+	+
Tetrahydrocortisone (17α-hydroxy-3α, 21-diacetoxy-pregnane-11, 20-dione)	E	375	−	+	−	−	+	+	+	+
11-Keto pregnanolone (3α-acetoxypregnane-11, 20-dione)	E	150	−	+	−	−	+	+	−	−
Compound S acetate (11-desoxycortisone acetate)	D, E, I	450	−	−	−	+	−	+	+	+
Desoxycorticosterone acetate (11, 17-desoxycortisone acetate)	B, F	375	−	−	−	+	−	+	−	+

*Refer to footnote * of Table III.

†Repeated tests on groups of five mice per test.

TABLE VII

STRUCTURAL ASPECTS OF STEROIDS INACTIVE AGAINST MOUSE LYMPHOSARCOMA*

Compound	Source†	Dose MG·/KG·/DAY	11-OXY	Δ^4-3-KETO	3-OH	17-OH	20-KETO	20-OH	21-OH
Testosterone	B	500	−	+	−	+	−	−	−
Progesterone	A, B, G	300	−	+	−	−	+	−	−
3β-Hydroxy-Δ^5-pregnene-20-one	G	300	−	−	+	−	+	−	−
$\Delta^{5,16}$-pregnadienolone	C	187.5	−	−	+	−	+	−	−
11-Desoxycorticosterone acetate	B, F	375	−	+	−	−	+	−	+
3β-Hydroxy-21-acetoxy-Δ^5-pregnene-20-one	B, G	375	−	−	+	−	+	−	+
17α-Hydroxyprogesterone (Δ^4)	B, I	250	−	+	−	+	+	−	−
17α-Hydroxyprogesterone (Δ^1)	B	250	−	+ Δ^1	−	+	+	−	−
17β-Hydroxyprogesterone	G	150	−	+	−	+	+	−	−
17α-Hydroxy-3β-acetoxyallopregnane-20-one	B	200	−	−	+	+	+	−	−
17α, 21-Hydroxyprogesterone acetate (Compound S acetate)	D, E, I	375	−	+	−	+	+	−	+
17α-Hydroxy-20β,21-diacetoxy-Δ^4-pregnene-3-one	C, D, I	300	−	+	−	+	−	+	+
17β-20, 21-Trihydroxy-Δ^4-pregnene-3-one	G	250	−	+	−	+	−	+	+
Δ^4-Androstene-3, 17-dione	B, C	375	−	+	−	−	−	−	−
Δ^1-Androstene-3, 17-dione	B	375	−	+ Δ^1	−	−	−	−	−
3β-Acetoxy-17α-hydroxy-Δ^5-pregnene-20-one	D	375	−	−	+	+	+	−	−
Δ^5-Androstene-3β, 17β-diol	B	375	−	−	+	+	−	−	−
21-Acetoxypregnane-3, 20-dione	B	500	−	−	−	−	+	−	+
3α, 12α, 21-Triacetoxypregnane-20-one	B	500	−	−	+	−	+	−	+
17β-Acetyl-$\Delta^{1,3,5}$-estratriene-3, 17α-diol	B	375	−	−	+	+	+	−	−
17β-Acetyl-$\Delta^{1,3,5}$-estratriene-3-ol	B	375	−	−	+	−	+	−	−
17α-Hydroxy-21-acetoxyallopregnane-3, 20-dione	B	500	−	−	−	+	+	−	+

*Repeated tests on groups of five mice per test.

†Refer to footnote * of Table III.

series. As the activities are relatively quite weak, the compounds require additional study to confirm the effects and extend them to other types of tumors.

In the tumor screening program it has not been feasible to make extensive detailed studies of effects on the host. Woolley, however, with

TABLE VIII

COMPARATIVE TUMOR AND ORGAN WEIGHTS IN ANIMALS RECEIVING CORTISONE BY DIFFERENT ROUTES

*Tissue**	*Subcutaneous*†	*Force Fed*†
Lymphosarcoma 6C3HED	0.2 gm.	1.1 gm.
Adrenals	0.013	0.020
Thymus	0.008	0.014
Spleen	0.30	0.31
Mesenteric lymph node	0.064	0.092
Liver	6.7	5.7

* Organ weights expressed in per cent of body weight less the tumor as measured on the tenth day.

† Dosage 1 mg./day/mouse in two doses.

TABLE IX

HEMATOLOGIC FINDINGS IN MICE TREATED WITH CORTISONE

	*Subcutaneous**		*Force Fed**	
Blood Cell Counts	*24 Hrs.*	*10 Days*	*24 Hrs.*	*10 Days*
Mean total RBC $\times 10^6$	9.6	4.6	10	7.2
Mean total WBC $\times 10^3$	3.1	3.7	3.5	5.4
Mean % lymphocytes	25	4	40	17
Mean % monocytes	6	2	5	6
Mean % neutrophils	68	94	53	76
Mean % eosinophils	1	0	2	0

* Dosage 1 mg./day in two divided doses per 20-gm. mouse, administered subcutaneously and orally, respectively.

selected steroids, has studied changes in the blood, organs, and tumors of mice bearing lymphosarcomas. He has reported[17, 24] that 11-oxygenated steroids with a strong antitumor activity depress the weight of the spleen to approximately one-third normal and of the thymus to one-tenth normal, decreasing significantly the size of the mesenteric lymph nodes, the adrenal cortex, and the pituitary. Cortisone appears to be more effective against the lymphosarcoma in the females than in the males. Tables VIII and IX show the effect of cortisone on organs and the changes in the blood. Woolley has studied the relative efficacy of subcutaneous and oral doses. The oral route is less efficient, though use of multiple doses increases the efficiency of this route. Sugiura, too, has observed the relative inactivity of single oral doses of cortisone in tumor tests. He has found

that intraperitoneal injections of saline suspensions also are relatively inefficient. Woolley has found that an 11-desoxy steroid, Compound L, at 500 mg./kg./day for ten days has revealed no tumor inhibition, no decrease in the lymphoid tissue, but a definite reduction in the adrenal cortex. These results with Compound L have not discouraged further trials of it and other 11-desoxy steroids.

Money[25] has studied selected steroids in normal rats for the effects upon certain tissue weights and the uptake of radioactive iodine. The changes observed with Compound L at two dose levels are shown in Fig. 6.

FIG. 6. Organ changes in rats injected with Compound L.

Compound L causes an increase in the adrenal weight, whereas cortisone-treated animals have much smaller adrenals. Cortisone produces an almost complete loss of thymic tissue, while Compound L causes less than half that decrease. Compound L increases markedly the lymph node weight, while cortisone destroys most of the lymph node tissue.

Bodansky and Markardt[26] have noted the influence of Compound L on plasma vitamin A levels of rats. These investigators further studied the interrelationship of vitamin A and Compound L in weanling rats on a vitamin-free diet. Groups were given different supplements of vitamin A. Differences were observed in the levels of vitamin A in the liver, kidney, and plasma of controls and animals receiving subcutaneous injections of Compound L. Particularly striking is the decreased level of plasma vitamin A in rats receiving injections of Compound L. On the basis of these studies Bodansky and Markardt have suggested that the interrelationship between steroid and vitamin actions be investigated further.

Summary

We have reported that certain steroids inhibit the development of the chick embryo. Of the many tested, a few 11-oxygenated steroids have been most inhibitory, but some of the 11-desoxy steroids have been effective in larger doses. Inhibitions of lymphosarcomas in mice by steroids have been demonstrated. In this activity, also, the 11-oxygenated steroids have been most active. Large doses of a few 11-desoxy steroids appear in preliminary trials to be weakly active.

Supplementary tests giving more detailed results with selected steroids have been described briefly.

The animal tests we have discussed cannot be taken as infallible, but they may give us leads insofar as certain types of neoplastic disease are concerned. We believe the screening tests against the mouse lymphosarcoma will prove useful in determining compounds likely to be active in human lymphoid tumors. It is realized that other test tumors are required in our tumor spectrum in order to find other types of antitumor activity. We are convinced that the steroids merit extensive screening not only against multiple types of tumors in experimental animals, but also in studies for miscellaneous types of biologic activity regardless of negative results in some of the standard steroid tests.

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Discussion

I. T. Nathanson: What are the effects of the active steroids on susceptible tumors if therapy is not initiated until a week or more after the tumor is transplanted and has established its growth capacities? The state of the tumor bed during the various phases of neoplastic growth may play an important role in the action of the steroids. For example, one must consider the vascular supply to the tumor as well as other changes in the stroma and tumor itself as growth progresses.

C. C. Stock: As Dr. Nathanson has revealed, he is familiar with the decreased effects of compounds on tumors after they have become established. Actually, with cortisone we have been able to obtain some inhibition of the lymphosarcoma after the tumor has grown for a week, but the effect is considerably less. Heilman and Kendall were able to show repeated inhibition of tumors in mice. I think with cortisone they actually saw some regressions. We have not seen regressions with cortisone.

R. Hertz: The only comment I would like to make is to supplement the data which Dr. Stock has offered on the prenatal chick with some observations we have on a specific tissue in the postnatal chick; namely the genital tract. In the immature chick the genital tract will have roughly this sort of an appearance (blackboard drawing), and weighs 20 mg. Under maximum dose of estrogen stimulation, over a period of eight days, that same tissue can be taken up to this size and weighs about 1000 mg. If such a dose of estrogen is accompanied by a maximally effective dose of progesterone, then we have a structure roughly about this size and it weighs around 300 mg. In other words, it is possible to inhibit estrogen-induced tissue growth in this specific tissue, with progesterone. Now, paralleling Dr. Stock's studies, we have screened by the above technique essentially the same series of steroid compounds which he showed you listed in his slides. Of those steroids we have found only the following to be active: first, progesterone, then, methyltestosterone, then Compound S, and allopregnane-21-ol-3,20-dione acetate. Of all the compounds tested, only these four were found to be active. Now these activities, as Dr. Lipschutz will bring out in his discussion, have no relationship whatsoever to the other biological effects of these particular steroids. When I present our data on the effects of progesterone on cervical cancer, I believe you will see why we are particularly interested in determining which of the steroids have this particular type of anti-estrogenic activity.

C. C. Stock: I want to express my appreciation for Dr. Hertz's emphasis on the variations in response due to differences in tissues or test conditions. I think it is very helpful to have this point.

A. White: What does Dr. Stock consider the effective dose of cortisone in suppressing growth of the osteogenic sarcoma as compared to the dose required to inhibit growth of the lymphosarcoma? Also, with respect to Compound S, the data indicated that S is inactive in the screening program at the Sloan-Kettering Institute, and yet in the conclusions I believe the suggestion was made that there was some activity. At one point in the presentation the statement was made that it had about one-twentieth the activity of cortisone. Will you clarify this point Dr. Stock, since I believe Dr. Hertz made the statement that his data on Compound S parallel those of Dr. Stock, and that S is quite active in the chick oviduct test?

C. C. Stock: With respect to the first question, the effective dose levels for the osteogenic sarcoma and the lymphosarcoma are about the same. The maximal effect that we have seen in the inhibition of those tumors is obtained with 37.5 mg. per kilogram per day for seven days, which is the maximum tolerated under these conditions. There was a slight titration indicated on the slide and you may have noticed that the activity against the osteogenic sarcoma does appear to decrease a little more rapidly than against the lymphosarcoma. With respect to Compound S, we have seen no activity with this compound in our program.

If I understood Dr. Hertz correctly, he saw his activity with Compound S in combination with progesterone. I believe that the confusion may have arisen in differentiating between 21-desoxycortisone and Compound S. We have not seen activity with Compound S but with 21-desoxycortisone, which appeared to be one-twentieth to one-fiftieth as active as cortisone. This is a very rough approximation because we haven't tried to titrate those comparisons accurately.

R. Hertz: The compounds that I listed on the board, i.e. progesterone, methyltestosterone, Compound S, and allopregnane-21-ol-3,20-dione acetate are the four that we have found to be active as depressors of estrogen-induced tissue growth. As I tried to point out, we are sometimes in agreement with Dr. Stock in his embryonic tissue-growth responses and at other points we find a difference, particularly with respect to Compound S. This compound is as active as progesterone in our inhibitory tissue-growth response while methyltestosterone is somewhat less effective, and the fourth compound is something between two and three times as effective as progesterone.

A. Segaloff: I notice that all the testing has been in transplantable tumors. We are all quite aware of the difference between tumors that have been transplanted for a long time, as most of these have, and in tumors as they appear spontaneously, or in their first transplant. I wonder if Dr. Stock

has any information as to whether spontaneous or first-generation transplant tumors differ in their reactivity to the various compounds as opposed to the ones that have been transplanted many times?

C. C. Stock: I can give you no information on this point insofar as the steroids are concerned because we've confined our tests to the transplanted tumors which have been through many passages.

K. Dobriner: Dr. Stock has clearly shown with certain steroids that the screening tests in animals provide important leads for study in patients with leukemia. On the other hand, it seems to me that additional screening procedures are necessary, since the present experimental tumor spectrum does not indicate a therapeutic possibility for testosterone and the estrogens. I wonder whether Dr. Selye or others have experience with screening tests for steroids which might offer leads for therapeutic trial in humans.

H. Selye: I am afraid I can't be of any help to Dr. Dobriner, but I would like to ask a few questions in connection with the effect of corticoids and other steroids on the chick embryo. My co-workers and I tested cortical extracts in 1940 when cortisone and other 11-oxygenated steroids were not yet available. We used the chick embryo, because we found the newly hatched chick was particularly sensitive to desoxycorticosterone acetate. By applying cortical extracts to the developing chick embryo we found a stunting of growth and a failure of the celiac cavity to close. (Dalton et al.: Canadian Med. Assoc., 1940.) In short, a syndrome similar to that described by Dr. Stock. However, there was also, as a rule, marked generalized edema and a malformation of the cornea in the sense of a keratoconus. I wonder whether Dr. Stock also observed such changes in his birds.

Secondly, I would like to know what Dr. Stock considers the immediate cause of death when too much cortisone is given to the mouse.

Finally, I would like to point to the extraordinary importance of Dr. Hertz's observation concerning pregnanedione. We were looking very carefully through all the literature on the pharmacology of pregnane derivatives at the time we published our four volumes on the pharmacology of steroids in 1943 and we couldn't find a single pregnane derivative which had any hormonal effects. Several of them, for instance, pregnanedione, had a marked anesthetic effect, but no hormonal effect. We concluded that only the allopregnane-etiocholane configuration or a double bond at C5 are compatible with hormonal activity. Other than the observation now reported by Dr. Hertz, I am unaware of any exception to this rule.

C. C. Stock: We have no definite observations on what causes death among the animals receiving the larger doses of cortisone. There may be persons in the audience who can contribute to this point.

We are giving rather large doses of cortisone and assume that some

of the complicated metabolic side-effects are causing the deaths. I had intended mentioning that we did notice edema in the chick embryo. I am not sure we noticed the eye lesion that you mentioned. Dr. Karnofsky has not called attention to it, but the histological studies have not been completed. The only thing noted grossly was the greater development of the eye with respect to the rest of the embryo. I might mention also that Landau had recorded that adrenal cortical extracts inhibited the development of the chick embryo. This point was brought out rather casually in one of his papers.

I'd like to comment just briefly on Dr. Dobriner's point. We certainly feel that the animal tumors we are employing may give us indications of the steroids that would be effective against leukemia or lymphosarcoma in man. The trouble is, we don't have enough active compounds that have been tested in both the mouse tumor program and in clinic to say whether there is going to be a good correlation. We expect there will be. Dr. Dobriner is quite right; other experimental tumors are going to be required to demonstrate the activities of compounds like testosterone. We have had quite an extensive screening program on other types of compounds in which over 4000 compounds have been tried. We did not start the steroids in this particular screening program against sarcoma 180 because we felt beforehand that it was not the type of tumor that would be likely to respond to steroid treatment. Therefore, we feel that for the different types of tumors which may be encountered in humans, you may have to look rather carefully to find a corresponding tumor in the animal and test it with compounds that are known to be effective against those tumors in the humans. The animal experimental screening programs and the clinical trials are mutually dependent.

A. Lipschutz: I have listened to the paper of Dr. Stock with the greatest interest, and also to the discussion of Dr. Hertz. The results are of considerable importance for the further development of the problem of steroids in the treatment of cancer. Dr. Dobriner asked a very important question: What is the program for further screening of steroids in the treatment of cancer? I think there is one relevant indication: The steroids which were active in preventing estrogen-induced abdominal fibroids were, in our work, 3-ketosteroids. We are fully convinced of this. Indeed, Dr. Iglesias in our department was successful also with pregnenolone-3-acetate, but on a quantitative level very different from that which is valid for 3-ketosteroids. Again, as Dr. Hertz has told us, he was successful in his work with the inhibition of estrogen-induced growth of the chicken oviduct when using a 3-ketosteroid. As far as I understood, the same rule applied to the inhibition of the growth of chick in the work of Dr. Stock. And finally, the same rule applies to steroids used in the treatment of cancer of the breast in humans. But there is one difference between our results and those of Dr. Stock. He has used quantities of about 12 to 37 mg. per day; on the contrary, in our work with the prevention of estrogen-induced fibroids, quantities about 1000 times less than that have

been used. There is indeed one very important point to be considered here: Much depends, in work with antitumorigenic steroids, upon the mode of administration of the latter. For instance, production of estrogen-induced abdominal fibroids can be prevented by, say 15 to 20 μg. of progesterone (micrograms, not milligrams!) per day when absorbed from subcutaneously implanted tablets. If progesterone is given by injections, one has to use much greater quantities. I may recall here the classic work of Courrier. The action of estrogen on the vaginal mucosa was shown to be counteracted, in the castrated rat, by quantities of progesterone about 200 times larger than the threshold quantity of estrone. On the contrary, in our work with subcutaneously implanted pellets of progesterone, prevention of estrogen-induced tumors was obtained with quantities of progesterone which were not greater than the tumorigenic quantity of the estrogen. This is why I would suggest to Dr. Stock to work with subcutaneously implanted pellets of cortisone. In collaboration with Drs. R. Iglesias and Elvir Mardones, we began recently work with cortisone in guinea pigs with estrogen-induced abdominal fibroids; I had to leave Santiago before definite results were available. But I may mention the following observation which is of interest. With progesterone or desoxycorticosterone acetate, certain anti-estrogenic actions can be observed: the estrogen-induced opening of the vaginal entrance is counteracted. In experiments with cortisone we have as yet not seen this anti-estrogenic action, at least not with the quantities used in our experiments.

C. C. Stock: I wish to thank Dr. Lipschutz for the suggestion of the use of the cortisone pellets. We have not tried this. There are so many problems that are connected with the route of administration and the question of absorption. It may be that some of our inactive compounds have not been active simply because they haven't been absorbed. I think one other point regarding the difference in required amounts of steroids is that there may be a different degree of sensitivity because you are using a system different from ours for measuring activity. Certainly the pellets should be tried from the viewpoint of the conservation of other steroids which might not be so readily available.

5

Antifibromatogenic Action of Steroids, With Special Reference to Pregnenolones

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Antifibromatogenic and Antitumorigenic Action of Steroids

When studying the antifibromatogenic action of steroids, the goal sought is knowledge of the *antitumorigenic* actions of these compounds. Subserous uterine fibroids have been induced with the continuous administration of estrogen in the mouse, rabbit, and guinea pig (Lacassagne, 1935, 1949; Nelson, 1937, 1939; Lipschutz and Iglesias, 1938; Iglesias, 1938). We found that fibroids can be induced easily in many other sites of the abdominal cavity of the guinea pig. Our findings have been corroborated subsequently by many authorities (see Von Wattenwyl, 1944; Mosinger, 1946; and the references given in Lipschutz, 1950, p. 6). These tumors have been used since 1938 in our work, in which the capacity of different steroids to prevent tumoral growth or to cause tumors to regress has been studied. Summaries of our work with the antifibromatogenic action of steroids have been given on former occasions (Lipschutz, Iglesias, Bruzzone, et al., 1948; Lipschutz, 1950). In this paper these summarized aspects shall be dealt with briefly, and emphasis shall be laid on some points of a more general interest, not discussed previously at length.

The Estrogen-induced Test Tumor

It is known that the estrogen-induced abdominal tumors are in general comprised of fibroblasts which produce abundant collagen fibers (Lipschutz, Vargas, and Iglesias, 1938; Lipschutz and Vargas, 1941a). Bundles of smooth muscle fibers also may be part of the structure of these tumors, and sometimes the tumors are fibromyomas or leiomyomas. The tumors shrink when the estrogen is withdrawn; in this respect their behavior is similar to that of uterine fibroids in women in the menopause. However, it must be kept in mind that shrinkage of the experimental fibroids—or of a uterine leiomyoma in the woman—does not mean disappearance, but only considerable diminution of size. This change is due

to the disappearance of the characteristic cellular component of the experimental fibroids, the fibroblast; the shrunken tumor consists almost exclusively of collagenous fibers with interspersed small nuclei.

Sometimes the experimental fibroid has a striking resemblance to the desmoid structures found in the human. (Compare, for example, Fig. 19 in Lipschutz, 1950 with Fig. 1 in Stout, 1948, and with Fig. 3 in Musgrove and McDonald, 1948).

The experimental fibroids, both the desmoid type and the fibrosarcoma, or the so-called "malignant tumor of fibroblasts" of humans (Stout, 1948), share with the spontaneous fibroids the faculty to infiltrate, engulf, and destroy surrounding tissues, e.g. the striated muscle of the abdominal wall, the smooth muscle tissue of the intestinal tract, and the pancreas. What has been said about spontaneous desmoid structures applies also to our experimental abdominal fibroids: "morphologically they have the local cancerous property of invasion, but histologically they are noncancerous" (Musgrove and McDonald, 1948). In a recent paper it has been suggested that estrogen-induced abdominal tumors of mesenchyme origin be called "desmoids" instead of "fibroids" (Nadel, 1950). However, this suggestion cannot be accepted, since only a limited number of these experimental tumors are structurally identical with desmoids. On the other hand, it has been suggested that the experimental estrogen-induced abdominal tumors be termed "fibrosarcomas" (Mosinger, 1946). But here again we feel that the more modest name of "fibroid" is more appropriate in order to distinguish it from the malignant tumor of fibroblasts in humans, which shows seemingly autonomous growth. This type of growth is not the case with the estrogen-induced experimental fibroid. Nor does the latter produce metastases which may occur, although rarely, with the malignant tumor in humans.

Thus it is evident that the estrogen-induced abdominal fibroid of the guinea pig is a tumor of a special kind, not completely identical with any type of spontaneous tumor in humans. Doubt has even been expressed that the abdominal fibroid is a tumor, because of its lack of autonomy (*Lancet*, 1946). This idea we must reject, not only for the sake of the prestige of our experimental abdominal tumor, but also for the case of several spontaneous tumors in humans, e.g. the benign uterine leiomyoma and the malignant and metastasizing prostatic cancer in its primitive stage. In these instances the tumor may regress when androgenic action is suppressed by castration or by administration of estrogen.

With the prolonged administration of estrogen, certain other highly interesting types of tumors of the mesenchyme also can be induced; for these the name "fibroid" is not appropriate. We have dealt more fully with these questions in our recent summary (Lipschutz, 1950, p. 13-16).

Advantages and Disadvantages of Work with the Estrogen-induced Test Tumors

The use of experimental abdominal fibroids in the guinea pig in work on the antitumorigenic action of steroids proved to be both an

advantage and a disadvantage. An advantage insofar as it permitted a very detailed experimental study of some fundamental details of the antitumorigenic actions of steroids; a disadvantage insofar as the antitumoral action of steroids certainly varies greatly depending on the type of tumor studied. It would undoubtedly have been of great importance to have examined different types of tumors. This is seen, for instance, in the success obtained by Heilman and Kendall (1944) with the latter's Compound E, now called "cortisone," in the treatment of a transplantable malignant lymphatic tumor of the thoracic cavity in the mouse; or by Murphy (1944) with testosterone propionate in preventing leukemia in mice; or by Lacassagne and Raynaud (1939) and by other authorities with testosterone propionate in preventing the hereditary adenocarcinoma in mice. It would have been more advantageous had we been able to try the antitumorigenic action of steroids with all these different types of tumors, in the same systematic way as was done with estrogen-induced abdominal fibroids. However, so extensive a program would have been beyond the capacities of a single research institution.

The Antagonism of Steroid Hormones

In our work with the antifibromatogenic action of steroids, we started in 1938 from our concept of the peripheral antagonism of sex hormones. This concept became established, after preliminary classical findings of Steinach, Knud Sand, and others, through our work approximately 25 years ago when there were no isolated and chemically identified female and male gonadal hormones, and when there was no knowledge whatsoever of steroids.

We worked then with the transplantation of the gonads into the organism of the other sex, under various experimental conditions. In animals with the ovary and testicle simultaneously present and both showing normal structures, characteristics of both sexes may attain full development. But cases also occurred in which characteristics of only one sex developed—the penis in the male, or the nipples in the female—even though the hormone-producing tissues of *both* sexes were present. To examine this dilemma we made the assumption that the action of the hormone of one sex on the respective peripheral organ is counteracted by the hormone of the opposite sex *at certain quantitative levels* (for references to this phase of our work—1925-1930—see Lipschutz, 1950, p. 125).

At a later time, when injectable hormones became available, the above assumption was corroborated by the work of different authorities. We mention only, from many similar experimental statements, the finding that the estrogen-induced epidermization of the vaginal mucosa in the castrated rat can be counteracted if, together with the female hormone, testosterone is also administered. This counteraction occurs provided the quantity of testosterone given is many times that of the estrogen (Courrier and Cohen-Solal, 1937).

There was so much unscientific discussion opposing this type of a direct antagonism between or among steroid hormones that the fact was

overlooked that from this concept of antagonism of hormones sprang the most outstanding discovery in the field of hormone therapy of cancer—the work of Huggins with estrogen therapy of prostatic carcinoma.

The Problem of Antitumorigenic Analogues

At this time no experimental knowledge exists as to the precise basis of the antagonistic action of antitumorigenic steroids. It will be useful here to refer to the remarkable work of Lacassagne and his associates with a similar antagonism in the field of carcinogenic hydrocarbons. These authorities succeeded in counteracting the cancerous effect of compounds of a highly carcinogenic potency by the simultaneous administration of compounds of a related chemical structure but of a relative low grade of carcinogenicity. In their work, the painting of the skin of mice with a solution containing both methylcholanthrene and the much less active dibenzfluorene diminished the carcinogenic action of the former hydrocarbon. Most spectacular results were obtained with the application of a solution containing the highly carcinogenic 9,10-dimethyl-1,2-benzanthracene and the noncarcinogenic 1,2-benzanthracene (Lacassagne, 1947, ch. v, pp. 110 and 112). The related compound of low activity acts evidently as an inhibitory analogue of the highly active hydrocarbon. Similarly, it has been suggested that antitumorigenic steroids may act as analogues of the tumorigenic steroids. In 1947, Segaloff suggested a trial of β-estradiol, a very weak estrogen, administered simultaneously with the potent fibromatogenic α-estradiol. When sufficient quantities of β-estradiol were administered (subcutaneously implanted pellets) during three months, the known toxic actions on the genital tract, e.g. increase of uterine weight, cystic glandular hyperplasia, invasive growth of the endometrial glands, and uterine bleeding, were elicited. However, in only exceptional instances was a fibromatogenic action evident, and in these cases it was insignificant in extent. This behavior of β-estradiol was similar to that we have formerly observed with other weak estrogens, which became fibromatogenic only when very considerable quantities were administered (Mello, 1944, 1945). No antifibromatogenic action was obtained when β-estradiol was administered simultaneously with α-estradiol. The results were unaltered whether the quantities of β-estradiol were smaller or greater than those of simultaneously administered α-estradiol (Iglesias, Mardones, and Lipschutz, 1950).

Antitumorigenic Actions Which Are Not Based on Antagonism

The significance of the antagonism of steroid hormones must not be overrated when their antitumorigenic action is discussed. Recognizing the action of estrogen in prostatic carcinoma, or of androgen in cancer of the breast, and possibly also in uterine fibromyoma, there is seemingly at the present time no basis for the concept of antagonism in the action of estrogen in cancer of the breast, or of progesterone and anhydrohydroxyprogesterone (ethinyl testosterone) recently also applied in prostatic

cancer (Trunnell et al., 1950), or of cortisone in lymphoid tumors (Pearson et al., 1949).

In certain other cases the antitumorigenic action of steroids is mediated by the anterior lobe of the hypophysis. In the castrated guinea pig with an ovarian graft in the spleen, a luteomatous condition develops at about ten months after grafting. This luteomatous condition is prevented, or counteracted, when progesterone, absorbed from a subcutaneously implanted pellet, is allowed to act in the animal for three months (Iglesias, Lipschutz, and Mardones, 1950). The luteomatous condition is not interfered with when a progesterone pellet is implanted in the ovary itself (Iglesias et al., unpublished data). In our work with antifibromatogenic steroids, mediation of the hypophysis is most probably out of the question since fibroids can also be elicited by estrogen in the hypophysectomized female guinea pig (Vargas, 1943).

Discontinuous and Continuous Action of Antifibromatogenic Steroids

We have referred to the faculty of testosterone to counteract the estrogen-induced epidermization of the vaginal mucosa. Progesterone also was known to have this activity, and progesterone was the first steroid we tried 12 years ago in our work with antifibromatogenic steroids.

When using injections of progesterone, quantities many times those of the estrogen were administered (Lipschutz and Vargas, 1941b). However, when subcutaneously implanted tablets or pellets of progesterone were employed, it was observed that relatively small quantities of progesterone were sufficient to counteract the fibromatogenic action of estradiol. Estrogen-induced abdominal fibroids can be prevented by quantities of progesterone equal to, or even smaller than, the quantities of estrogen simultaneously absorbed from a subcutaneously implanted pellet (Lipschutz, Bruzzone, and Fuenzalida, 1944).

There is another relevant fact: the quantity of estrogen may vary greatly without interfering with the antifibromatogenic action of progesterone (Lipschutz et al., 1944).

The comparative quantitative aspects of the antifibromatogenic action of progesterone administered by injection versus that of progesterone absorbed from subcutaneously implanted pellets are of considerable interest both from an experimental and clinical standpoint. Fibromatogenic action of estrogen has been shown by our work to be dependent on *continuous* action of the hormone; it is by continuous action, rather than by the rhythmical or intermittent type, that the hormone acquires tumorigenic faculty. Large quantities of estrogen, for example, a total of 10 mg. of estradiol in three months, have to be injected in order to elicit abdominal fibroids. On the other hand, when using a slowly absorbed ester of the type of Miescher's 17-caprylate of estradiol, a total of only 0.2 mg. of estradiol in three months, i.e. 50 times less, is sufficient to produce the same, or a greater, tumoral effect. Small quantities of the free estradiol absorbed continuously from a subcutaneously implanted pellet produce

a similar effect. Thus, continuous absorption of the antifibromatogenic steroid from a subcutaneously implanted pellet, in contrast to administration by injection, is of fundamental importance.

Subcutaneously implanted pellets have been also used in our work with the *treatment* of estrogen-induced fibroids (Lipschutz and Maass, 1944; Lipschutz and Schwarz, 1944).

Comparative Antifibromatogenic Action of Different Steroids

Subcutaneously implanted pellets were also used in our work with the antifibromatogenic action of other steroids. The necessary range of absorption was obtained by varying the number of pellets implanted. In order to obtain absorption of very small quantities, the steroid was mixed with cholesterol; in this way, absorption of the steroid decreases very considerably, indeed beyond that expected on the basis of the diminished surface the steroid occupies in the pellet (Fuenzalida, 1950).

Testosterone propionate also was found to be antifibromatogenic; however, with this steroid, quantities many times those of progesterone were necessary to obtain the antifibromatogenic effect. Desoxycorticosterone acetate also is antifibromatogenic; it is less active than progesterone but more active than testosterone. In this phase of our work the question arose where the antifibromatogenic action is related to the known physiologic activities of these steroids. The question also arose as to the structural differences of antifibromatogenic steroids. In the course of this work 24 different steroids and 5 steroid esters were studied.

In some of this comparative work a method was used which seems to be of a rather general interest. When a metallic tablet, i.e. the dental alloy of aluminum and copper, is introduced into the abdominal cavity, it becomes attached to the epiploon, and more rarely to other parts of the peritoneum; then it is surrounded by a thin capsule. This is simply a foreign-body reaction. When estrogen is given, the capsule becomes thickened, or tumoral. Similar behavior is observed with a glass bead in the abdominal cavity.* The estrogen-induced tumoral thickening of the capsule is counteracted by progesterone but not by those steroids which are devoid of antifibromatogenic activity (see Manhood, Monreal, Bruzzone, Schwarz, etc., in summary of Lipschutz, 1950, pp. 49-51, and pp. 143-146).

Structural Particularities of Antifibromatogenic Steroids

The question of an interrelationship between the chemical structure of steroids and their physiologic actions has been studied in recent times especially by Selye (1942) and his associates. We shall refer here to this question only briefly, and only insofar as it is related to our work with the antifibromatogenic action of steroids.

* In work of Bruzzone and Kliwadenko still in progress, Permutit powder (see the Merck Index, 5th edition, 1940, p. 418) was introduced into the abdominal cavity of guinea pigs so as to cause a more generalized mechanical stimulation, and later on estrogen also was administered (subcutaneously implanted pellets). A very considerable tumoral effect was obtained in most of these animals. Permutit alone produces an adhesive fibrosis but no tumors.

The first point to attract attention was the fact that the three mentioned antifibromatogenic steroids, progesterone, desoxycorticosterone, and testosterone, were 3-keto compounds. We tried various compounds with a hydroxyl group at C3; none of these compounds showed antifibromatogenic action when tested in quantities comparable to the mentioned 3-keto compounds.

Since the antifibromatogenic activity of progesterone and desoxycorticosterone acetate is superior to that of progesterone propionate, the question arises as to whether the side chain of two carbons at C17 plays a role and, consequently, whether pregnenolones will share antifibromatogenic activity with progesterone and desoxycorticosterone. No antifibromatogenic action was obtained with pregnenolones as long as we worked with quantities comparable to those of progesterone or desoxycorticosterone (Lipschutz, Bruzzone, and Fuenzalida, 1943). However, when very large quantities were given, pregnenolones also showed antifibromatogenic activity (Iglesias and Bruzzone, 1948). We shall deal with this question in a separate section.

The antifibromatogenic steroids have a double bond, Δ^4, in ring A. But dihydrotestosterone is no less, if not more, antifibromatogenic than testosterone. We then hopefully tried pregnanedione and allopregnanedione; the results were negative.

It would probably not be an exaggeration to say that the study of the comparative antifibromatogenic action of different steroids from the point of view of structural peculiarities did not lead to any new insight or understanding of this type of action of steroid compounds.

Antifibromatogenic Action Is Not Dependent on Progestational Activity of Steroids

Let us now refer to the question of whether the antifibromatogenic action is related to the known physiologic activities of the respective steroid compounds.

The antifibromatogenic action of steroids decreases with their diminishing progestational activity (progesterone → desoxycorticosterone → testosterone). Consequently we assumed, years ago, that antifibromatogenic activity is correlated, or associated, with the progestational property (Lipschutz, 1944). The fact that the nonprogestational Δ^{16}-dehydroprogesterone was not antifibromatogenic seemed to be in favor of our assumption. However, subsequent work showed the fallacy of this deduction. It is known that the progestational activity of testosterone is increased by substitutions at C17 (Klein and Parkes, 1937). But the antifibromatogenic action of these compounds was found to be not greater, and often even smaller, than that of progesterone (Lipschutz, 1946; Lipschutz et al., 1948).

The dissociation of antifibromatogenic action from the progestational property may be exemplified by new comparative work with two steroids. Ethinylandrostenediol (EA) has been shown by Masson and Selye (1945) to be progestational; its activity is not inferior, if not superior, to that of

methyldihydrotestosterone (MD). A comparative study of the antifibromatogenic action of these two compounds offers an excellent opportunity to examine the question of correlation or noncorrelation of progestational and antifibromatogenic actions. Androstenediol is almost devoid of progestational action (Selye and Masson, 1943); it also lacks antifibromatogenic action (Iglesias, unpublished). Will a substitution at C17, by which androstenediol becomes progestational, confer on this compound antifibromatogenic activity also?

The results of our comparative study (Iglesias, Mardones and Lipschutz, in press) are summarized in Table I.

Pellets of estradiol, and of ethinylandrostenediol or methyldihydrotestosterone, were implanted simultaneously beneath the skin of castrated female guinea pigs. The fibrous tumoral effect (FTE) was classified in arbitrary units (Lipschutz, Vargas, et al., 1941; Lipschutz and Maass, 1944). Fibroids were not prevented even with as much as 205 to 430 μg. of ethinylandrostenediol per day. On the other hand, the antifibromatogenic action became evident with about 100 μg. of methyldihydrotestosterone and was definite with about 160 μg. per day. Methyldihydrotestosterone also counteracted the extreme growth of the uterus and prevented uterine bleeding as induced by estrogen; none of these actions was manifest with ethinylandrostenediol.

These results substantiate and extend the conclusions which were reached in our former work with androgens with substitutions at C17, namely,

1. that acquisition of progestational activity does not confer antifibromatogenic properties; and
2. that compounds having the same progestational activity may differ greatly as to antifibromatogenic activity.

Antitumorigenic Action of Steroids—An "Independent" Property of These Compounds

What has been shown for the noncorrelation of antifibromatogenic and progestational actions has been extended also to the lack of relationship of antifibromatogenic and androgenic actions, and likewise of antifibromatogenic and corticoid actions (Lipschutz, 1950, ch. 13). The new results referred to above substantiate the conclusions we had reached in 1946, i.e. that antifibromatogenic action is a property *per se*, not a consequence of, any known or classic physiologic action of the respective steroids. Antifibromatogenic action is an "independent" property of these compounds, not "subordinate" to any other action.*

We feel that this conclusion, drawn from purely experimental work, is of considerable relevance for all further clinical trials of the antitumorigenic action of steroids. This can best be illustrated by some new developments in the hormonal treatment of cancer of the breast. Testosterone propionate has been used hitherto, with the exception of those cases in

* Work with methylandrostenediol, with *cis*-testosterone and cortisone also is in progress.

TABLE I

COMPARATIVE ANTIFIBROMATOGENIC ACTION OF EA (ETHINYL-Δ^5-ANDROSTENEDIOL; M. P. 243°) AND MD (METHYLDIHYDROTESTOSTERONE; M. P. 191°)
DURATION OF EXPERIMENTS: 3 MONTHS

Series	*Number of Animals*	*Estradiol per Day µg.*	*EA or MD per Day µg.*	*Number of Animals with Genital Bleeding*	*FTE* Average and Range*	*Number of Marks 2 and 3 Average*	*Uterine Weight Average and Range gm.*
CXXVI	8	28– 52	0	1	3.8 (0.5– 6.0)	1.0	4.4 (3.1–6.4)
			EA†				
CXXVI	10	27– 52	12– 59	0	4.4 (1.0– 9.5)	1.5	3.6 (2.0–6.1)
CXXVI	5	91–113§	153–185	1	5.5 (1.5– 9.0)	1.5	3.5 (3.0–5.0)
CXXVI	3	75–102§	205–257	1	6.8 (2.5–12.0)	2.3	4.4 (2.0–7.0)
CXXVI	2	95– 99§	344–450	0	7.5 (3.0–12.0)	2.0	4.5 (3.1–6.0)
			MD‡				
XCVII	5	25– 41	11– 15	0	4.3 (2.0– 7.0)	1.0	4.2 (2.3–6.0)
XCVII	6	29– 73	74–119	0	2.6 (1.0– 5.5)	0.7	3.7 (2.9–4.2)
XCVII	7	31– 56	146–179	0	1.7 (1.0– 3.0)	0.2	3.9 (2.7–4.7)
XCVII	3	41– 48	223–293	0	0.8 (0.5– 1.0)	0	2.5 (2.3–2.8)
XCVII	3	32– 43	333–453	0	0.8 (0 – 1.5)	0	2.0 (1.4–2.9)

* FTE = fibrous tumoral effect.

† Number of pellets implanted: 1 to 6. Absorption per sq.mm. of the pellet was of 0.6 µg. (average).

‡ Number of pellets implanted: 1 containing 40% of MD, ½ to 2 of pure MD. Absorption per sq.mm. of the pellet was 2.7 µg. (average).

§ Average fibromatogenic action is probably increased with large quantities of estradiol. But antifibromatogenic action is not interfered with by variation of quantity of estrogen simultaneously administered (experiments with progesterone; Lipschutz, Bruzzone, and Fuenzalida 1944), or by the chemical nature of the estrogen (Lipschutz and Grismali 1944; Bruzzone 1949). See also the important statements of Hertz et al. (1947) in experiments with the growth promoting action of stilbestrol on the oviduct of chicken. The preventive effect of progesterone was not altered when the estrogen dosage was increased as much as 16-fold!

which estrogen was applied. But testosterone offers the great disadvantage of producing virilizing effects in women. Recently trials were made in breast cancer with methylandrostenediol (Homburger et al., 1950). Its androgenic, or virilizing, action was claimed to be insignificant compared to that of testosterone and some of its C17 derivatives. But methylandrostenediol shares, seemingly, with testosterone some of the beneficial effects of the latter in breast cancer (Homburger et al., 1950). We feel that further clinical work along these lines would be fundamental.

Diversified Actions of Pregnenolone

Let us now discuss questions related to pregnenolone.

Mention has been made of the fact that the antifibromatogenic activity of steroids is intimately related to the ketonic group at C3. Steroids with a hydroxyl group at C3 are devoid of antifibromatogenic activity when quantities comparable to those of progesterone or desoxycorticosterone are given. The fact also was mentioned that pregnenolones may show antifibromatogenic activity when very large quantities are given. Whereas these results do not necessarily alter the concept that the ketonic group at C3 is essential for the antifibromatogenic activity of steroid compounds, both pharmacologic and clinical interest is attached to these findings with pregnenolone.

Statements regarding the manifold actions of pregnenolone have been summarized recently in a very careful manner by Henderson et al. (1950) and may be classified under two headings:

First: Pregnenolone when administered in large quantities manifests unexpected and very diversified activities: progestational, corticoid, spermatotrophic, and lactogenic. These actions may be, seemingly, also contradictory; pregnenolone imitates certain androgenic actions but also estrogenic ones, e.g. enlargement of the preputial glands in the rat, in any case in females, and cornification of the vaginal mucosa; but pregnenolone may also show anti-estrogenic action in preventing the decline of testis weight induced by estrogen.

Second: Pregnenolones are completely devoid of toxic actions even when given in large quantities.

It would be idle to speculate about the mechanisms which may underlie activities so diversified as estrogenic, androgenic, anti-estrogenic, progestational, gonadotrophic, etc. An older and spectacular statement of Deanesly and Parkes (1936) may be recalled here: Very large quantities of testosterone, or other androgens, administered to castrated mice can elicit evidence of estrogenic action. The explanation of the fact that so diversified and contradictory activities are produced by steroid compounds, when given in quantities probably much greater than the hormonogenic potentialities of the organism in health or disease, may be based on two different possibilities: first, stimulative actions of the steroid compound on different hormone-producing organs; and second, the metabolic fate of the steroid by which the latter is transformed into another steroid of different and even opposite activity. The metabolic fate

may be dependent on the quantity administered, or on the functional condition of the organism. One must recognize that at this moment it seems impossible to disentangle these fascinating problems of steroid activity. But it would seem worthwhile to approach them experimentally, with special reference to pregnenolone which seems to show activities more diversified than any other group of steroids, though this might be due to no other steroid having been administered so far in such large quantities.

Antifibromatogenic Action of Pregnenolones

The antifibromatogenic and antitumorigenic actions of steroids are at the present time before us as a definite problem of experimental pathology. In view of so many unexpected actions of pregnenolone, it may be of interest to summarize the work we have done with large quantities of 21-acetoxypregnenolone (AOP) and pregnenolone acetate (PA), even though this work has as yet not been extensive.

As seen from Table II, quantities of 21-acetoxypregnenolone, up to about 15 times those of the antifibromatogenic progesterone threshold, were unable to prevent estrogen-induced fibroids: with as much as 79 to 362 μg. of 21-acetoxypregnenolone no significant decrease of the fibrous tumoral effect was obtained. No importance can be attributed to a "decrease" from fibrous tumoral effect of 5.1 to fibrous tumoral effect of 4.1 as seen in the group receiving 279 to 362 μg.; even in a control group, fibrous tumoral effect may be as little as 4, or somewhat less, as seen for example in Table I.

There was still a remarkable tumoral effect even with as much as 808 to 1241 μg. of 21-acetoxypregnenolone (Figs. 1 and 2). But the fibrous tumoral effect seems definitely to diminish at this quantitative level, although uterine weights are still of the same range as in control animals.

With pregnenolone acetate, the antifibromatogenic action was more pronounced than with 21-acetoxypregnenolone, as shown by data in Table III.

The antifibromatogenic action of pregnenolone acetate becomes noticeable when quantities as large as approximately 800 μg. per day are absorbed from subcutaneously implanted pellets (Fig. 3).

Estrogen-induced uterine growth was scarcely influenced by large quantities of 21-acetoxypregnenolone (Table II, last column); however, with pregnenolone acetate there was rather considerable prevention of uterine growth (Table III). Uterine bleeding was not prevented with large quantities of 21-acetoxypregnenolone; it was, however, prevented with pregnenolone acetate.

Neither of the two pregnenolones prevented estrogen-induced growth of the mammary glands. However, it may be recalled here that this inhibition was not obtained in the guinea pig with any of the antifibromatogenic steroids. Mammary development was seemingly greatly stimulated by simultaneous administration of either 21-acetoxypregnenolone or pregnenolone acetate with estrogen, particularly in the case of pregnenolone

TABLE II*

COMPARATIVE ANTIFIBROMATOGENIC ACTION OF PROGESTERONE (P) AND 21-ACETOXYPREGNENOLONE (AOP) (M. P. 183°)
DURATION OF EXPERIMENTS: 3 MONTHS

Series	*Number of Animals*	*Estradiol per Day µg.*	*P or AOP per Day µg.*	*Number of Animals with Genital Bleeding*	*FTE Average and Range*	*Number of Marks 2 and 3 Average*	*Uterine Weight Average and Range gm.*
XLVII and XCVIII	25	16–46†	0	7	5.1 (1.0–10.0)	1.7	5.3 (2.6–10.7)‡
			P				
XLVII	14	28–56	13–24‖	0	1.4 (1.0– 2.5)	0	3.0 (1.7– 5.1)
			AOP				
XLVII	15	21–63	79–362¶	2	4.8 (1.0– 8.0)	1.4	4.3 (2.5– 8.2)
XCVIII	14	30–55§	808–1241**	0	3.0 (1.5– 5.5)	0.9	4.2 (2.0–10.6)
			AOP				
XLVII	5	26–63	19–116	1	5.8 (2.5– 8.0)	1.8	5.2 (4.0– 8.2)
XLVII	5	30–40	144–228	0	4.5 (1.5– 7.5)	1.4	4.2 (2.5– 6.4)
XLVII	5	21–37	279–362	1	4.1 (1.5– 6.5)	1.0	3.6 (2.5– 6.2)

* This table contains results given in Lipschutz, Bruzzone, and Fuenzalida (1943) and Iglesias and Bruzzone (1948).
† Partly with estradiol dipropionate.
‡ For 10 animals only.
§ With estradiol dipropionate.
‖ With 5 to 12 µg. of progesterone per day the antifibromatogenic effect was nil or only weak. See Lipschutz, Bruzzone, and Fuenzalida (1943).
¶ Number of pellets implanted: 1 to 4.
** Number of pellets implanted: 10 and 12. Absorption per sq.mm. of the pellet was 1.4 to 1.7 µg. (average 1.5 µg.).

FIG. 1. Antifibromatogenic action of *small* quantities of progesterone absorbed from subcutaneously implanted pellets (Table II). Left: Average and maximal reaction in a group of animals receiving simultaneously estradiol (E) and progesterone (P). Absorption per day: 30 and 36 μg. of E; 13 and 16 μg. of P. Right: Average and maximal reaction in a group of animals receiving only estradiol. Absorption per day: 29 and 16 μg. of E per day.

acetate. The mammary gland may extend on a considerable surface in similar animals with pregnenolone acetate; the alveoli may be greatly distended by secretion to a degree not seen generally with estrogen alone. On the other hand, pregnenolone acetate when given alone without estrogen produces no mammary growth whatsoever.

TABLE III*

ANTIFIBROMATOGENIC ACTION OF PA (PREGNENOLONE ACETATE; M. P. 149–150°)
DURATION OF EXPERIMENTS: 90 TO 99 DAYS

Series	*Number of Animals*	*Estradiol per Day µg.*	*PA per Day µg.*	*Number of Animals with Genital Bleeding*	*FTE Average and Range*	*Number of Marks 2 and 3 Average*	*Uterine Weight Average and Range gm.*
CXXXI	10	15– 44† 51– 79	0	8	6.4 (1.5–10.0)	2.0	6.5 (2.5–14.5)
LXXXI	19	36– 72	114– 506‡	0	5.2 (0.5–10.0)	1.8	4.8 (2.5–13.6)
CXXXI	10	14– 34 32–103	820–1580§	0	1.3 (1.0– 3.0)	0.1	2.1 (1.4– 2.9)
CXXXI	4	14– 34 32– 96	820– 980	0	1.1 (1.0– 1.5)	0	1.9 (1.4– 2.2)
CXXXI	6	18– 33 40–103	1040–1580	0	1.4 (1.0– 3.0)	0.2	2.3 (1.7– 2.9)

* The present table contains part of the results in Iglesias and Bruzzone (1948). Various animals with low estrogen absorption have been omitted.

† First line: absorption per day during 34 days. Second line: absorption per day during the last 62 days.

‡ Number of pellets implanted: 1 to 4.

§ Number of pellets implanted: 8 to 12. Absorption per sq.mm. of the pellet was 1.5 to 2.8 µg. (average 2.2 µg.).

It seems evident that pregnenolone acetate is more antifibromatogenic than 21-acetoxypregnenolone. This gives additional proof that antitumorigenic action is not subordinated to known physiologic actions of steroids since corticoid activity of pregnenolone acetate has been shown to be considerably less than corticoid activity of 21-acetoxypregnenolone (Bruzzone et al., 1946; Bruzzone and López, 1948).

As already mentioned, the fact that large quantities of pregnenolone may show antifibromatogenic action cannot be taken as a proof against our former conclusion that the ketonic group at C3 is fundamental for the antifibromatogenic action of steroids. This is justified in the same

FIG. 2. Average and maximal reaction in a group of animals receiving simultaneously estradiol and *large* quantities of 21-acetoxypregnenolone (AOP; Table II). Absorption per day: 41 and 30 μg. of E; 1160 and 998 μg. of AOP.

way that the statement of Deanesly and Parkes mentioned previously; that large quantities of testosterone are estrogenic does not shake the law that estrogenic action is a specific property of a compound characterized by certain peculiarities. Many more steroids would most probably reveal the antifibromatogenic action when administered in quantities as large as those used in our work with the pregnenolones. But from the point of view of clinical application, pregnenolones offer greater interest because of their nontoxicity.

Pregnenolone Is Not Toxic in the Guinea Pig

There were no noticeable toxic or other unexpected actions in our guinea pigs which could have been attributed to the large quantities of pregnenolone acetate which were administered during three months by continuous absorption from subcutaneously implanted pellets (Table III).

FIG. 3. Antifibromatogenic action of *large* quantities of pregnenolone acetate (PA; Table III). Left: Average and maximal reaction in a group of animals receiving simultaneously estradiol and PA. Absorption per day: 22 to 32 μg. and 18 to 40 μg. of E; 980 and 1300 μg. of PA. Right: Average and maximal reaction in a group of animals receiving only estradiol. Absorption per day: 33 to 68 μg. and 23 to 71 μg. of E.

However, in the above work, pregnenolone acetate was administered together with estrogen, and for this reason an additional experiment may be quoted in which pregnenolone alone was given to castrated female guinea pigs. The data are presented in Table IV.

Body weight was not impaired. No estrogenic or androgenic actions were observed. There was, as already mentioned, no growth of the nipples or of the mammary glands. The vaginal entrance remained closed for the whole duration of the experiment. Uterine weight was, with up to about 800 μg. of pregnenolone acetate, the same as in castrated animals. A slight increase of uterine weight possibly took place with quantities above 1000 μg. per day. In one animal with 1005 μg. of pregnenolone acetate per day, uterine weight reached 1.3 gm. and a small growth of the nipples took place. This estrogenic action might have been due to an ovarian remnant which is sometimes involuntarily left in the body; however, even omitting this animal, the average in the group with larger quantities of pregnenolone acetate was 0.5 gm. instead of 0.3 gm. as in

TABLE IV

ADMINISTRATION OF PREGNENOLONE ACETATE TO CASTRATED FEMALE GUINEA PIGS
DURATION OF EXPERIMENTS: 92 DAYS

Series	*Number of Animals*	*PA per Day μg.*	*Body Weight Average and Range*		*Vag. Opening*	*Growth of Nipples*	*Uterine Weight Average and Range gm.*
			Initial gm.	*Final gm.*			
CXXXVIII	5	512–785*	500 460–530	640 540–740	0	0	0.3 0.2–0.4
CXXXVIII	6	1005–1185*	490 350–570	613 410–750	0	0	0.6 (0.5)† 0.3–1.3 (0.3–0.7)†

* Number of pellets implanted: 8 to 12.
† When omitting the animal with 1.3 gm. uterine weight (see text).

the group with smaller quantities. In the series of Table IV, there was no increase of the clitoris suggestive of androgenic action; this finding is significant, since among the animals listed in Table III there were several with a noticeable, though small, growth of the clitoris (Iglesias and Bruzzone, 1948).

The absence of noticeable toxic actions and the lack of estrogenic or androgenic effects in these animals receiving pregnenolone acetate is of particular interest since the quantities given were as large as 1.0 to 1.5 mg. per day, or 2 to 3 mg. per kg. of body weight, administered during three months (see Tables III and IV). This corresponds to about 130 to 200 mg. per day in humans. Moreover, it must be kept in mind that this administration was by continuous absorption from pellets, a procedure which, in general, yields a greater potency of steroids than is usually obtained with injections.

Some General Conclusions

Experimental results obtained, on the one hand, with antifibromatogenic action of various steroids, and the possible clinical results obtained,

on the other hand, in the treatment of cancer with the same compounds, or with stilbestrol instead of the natural estrogen, must serve as a potent stimulus for further research in this field. However, to the present time, clinical application has certainly been limited.

When trying to apply experimental results with animals to the hormonal treatment of tumors in humans, one meets a fundamental difficulty. Just as a homologous structure, e.g. the mammary gland, responds to the tumorigenic stimulus of estrogen differently depending on the species or strain used, so also the response to the antitumorigenic steroids may differ in two species. It suffices to mention that testosterone, which has been shown to be effective in the treatment of breast cancer in humans, is ineffective in counteracting the stimulative action of estrogen on the mammary gland in the guinea pig, or in counteracting metaplastic mammary changes which take place in the mammary gland with the prolonged administration of estrogen (Lipschutz, 1950, ch. 16).

However, one must be careful not to underrate experimental work of this type solely because of these species differences in the response to antitumorigenic steroids. Our task in experimental research is not only to discover those steroids which may serve in the hormonal treatment of cancer, but also to contribute to the knowledge of the laws which underlie antitumorigenic actions of steroids. It is also from this point of view that results of experiments made in guinea pigs, rats, or mice have to be evaluated.

Experimental results of studies of antifibromatogenic action of steroids on estrogen-induced abdominal fibroids may be summarized in the following conclusions of particular interest to the clinician:

1. certain 3-keto compounds among the steroids are able to prevent estrogen-induced abdominal fibroids; progesterone has been shown to be the most potent antifibromatogenic steroid, whereas various other 3-keto compounds are devoid of any antifibromatogenic activity;

2. hitherto it has not been possible to give a satisfactory explanation of this comparative antifibromatogenic activity of 3-ketosteroids from the point of view of particular aspects of their chemical structure;

3. antifibromatogenic action of steroids is not related to their known physiologic activities, e.g. progestational, corticoid, or virilizing actions;

4. antifibromatogenic action is an independent property of certain steroids;

5. a steroid having antifibromatogenic action may be ineffective in preventing estrogen-induced epithelial proliferation, or metaplasia, of other tissues such as the mammary gland;

6. the fact that a steroid acts as an antitumorigenic compound on a tissue of one species does not mean that it will be able to act in a similar manner on the same tissue in another species. This is seen from the differential behavior of testosterone in cancer of the breast in humans as compared to abnormal mammary proliferation in the guinea pig;

7. antifibromatogenic action can be obtained with minute quantities

of certain 3-ketosteroids, provided that absorption is a continuous one, as provided by subcutaneous implantation of pellets;

8. when quantities sufficiently large are given, antifibromatogenic action may also be obtained with steroids having a hydroxyl group at C3, for example, pregnenolone. The quantities necessary are many times larger than those of progesterone; and

9. no toxic effects have been seen in guinea pigs receiving as much as 1 to 1.5 mg. of pregnenolone acetate per day by continuous absorption from subcutaneously implanted pellets; this corresponds to approximately 130 to 200 mg. per day in humans.

Paragraphs 5 and 6 indicate that it would be impossible to recommend antitumorigenic steroids to the clinician solely on the basis of experiments in animals. On the other hand, paragraphs 1 to 4 suggest that the clinician should make a very ample and systematic search for antitumorigenic steroids. Paragraphs 3 and 4 give evidence that this search has to be made not only among those steroids present in the body, and not only among those steroids which may exhibit some of the known physiologic actions. A steroid devoid of any of these actions may be effective in counteracting tumoral growth. Paragraphs 8 and 9, referring to pregnenolone, suggest that such a steroid might be found even among steroid compounds which do not have the specifications indicated in paragraph 1.

One must look forward hopefully to the work of the clinician for the application of steroids in cancer.

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Discussion

I. Gonzalez-Guzman: The structural scheme of the nucleoli shows two different components: the fundamental substance and the granular apparatus and its visualization is made possible by a convenient modification of del Rio Hortega's silver double staining method. According to the ideas of Cassperson, the production of ribonucleic acid and proteins rich in hexonic bases is governed by the "associated chromatin," a chromosomic substance with genetic activity. According to my own ideas, it is not the associated chromatin that makes the proteins but it is the fundamental substance of the nucleoli that is able to produce nucleic acids and proteins rich in hexonic bases. The granular apparatus may conduct the synthesis of lipoprotein material. After many years of investigation in cytophysiology and on the same subject of this symposium, I am convinced that this is true. I am convinced, also, of the lipid production by some cells, for example, those of the adrenals and of the "theca interna" of the ovary.

The cells of the fascicular and plexiform zones of the adrenals, like those of the "theca interna" at the very early stages of the maturation of the yellow body, show large nucleoli and a high content of silver-staining granules. These nucleoli are the richest of the whole body in granular formations. This fact suggests their very important role in lipoprotein formation in the adrenal cortex and in the yellow body.

Because of the very short time allowed for this discussion, it is not possible for me to give you a description, or even a simple résumé of my research and findings on this subject. I did show, before the Eighth Scientific American Congress held at Washington, D. C. in 1940, that cancerous cells produce important amounts of lipids, and this fact is in close relation with a highly developed nucleolar apparatus enriched in silver-staining granules. In some cases these granules left the nucleoli and migrated to the nucleolar border.

In his very important paper, Dr. Lipschutz showed the existence of some hormones capable of increasing the growth of certain tumors and the existence of others which inhibit tumor growth. It will be important and profitable to study the nucleolar apparatus of the adrenal cells and those of the tumors because it may be possible to establish some relations

between the chemical structure of the compound assayed and the magnitude of the cellular response as evaluated by modifications of the nucleolar apparatus.

A. R. Abarbanel: Dr. Lipschutz' excellent presentation raises several very basic questions, particularly as they refer to the steroids. In the first place I think the time has come, as I have said in the past 11 years, that we regard all the steroids primarily as chemical substances which exert constitutional effects. Let us forget, if we can, that these compounds are so-called "sex" hormones. Testosterone cannot make a male, nor estradiol a female. We call salt "sodium chloride"—then let's call a particular steroid derivative by its chemical name. Thus, I am sure that there won't be the great misunderstanding that exists. There is one universal metabolic system or series of systems.

In the second place, the fibroids or fibromyomas that Dr. Lipschutz has developed in his very beautiful fundamental experiments along those lines have been translated wrongly into the clinical picture of fibroids or fibromyomas in the human. We were told that by giving estrogens in humans we might produce fibroids. This has not proved to be correct. Then many attempted to use progesterone to shrink this growth in the human. We have a large series of cases of myomas in pregnancy. At about the third or fourth month these myomas begin growing very rapidly and continue to grow until about the sixth or the seventh month. Very frequently it is found that following pregnancy, the tumor may regress; sometimes one cannot find them. In other words, there is something during pregnancy that makes them regress. On the other hand, we often run into a syndrome called "acute red degeneration." This syndrome is caused by the fact that there is a single arteriole which feeds the myoma while the veins are multiple. This arteriole becomes thrombosed. In the acute state, the myoma is red and then it will gradually undergo hyaline and fatty degeneration and may even become calcified. Now we felt originally from Dr. Lipschutz' work that if we gave large doses of progesterone we could help solve the problem. But this was not so. Instead, we have found that we cannot only relieve the pain but we can also prevent these women from aborting, which most of them do, by giving them very massive doses of diethylstilbestrol. In the average case of acute red degeneration of a myoma, as much as 1.0 gm. per day of hormone for weeks may be required to control the pain and prevent miscarriage. At first we were concerned that the myoma might be made larger, but this was not the case. It is, of course, very difficult to measure the size of these myomas in pregnancy. From clinical evidence, and it is merely an impression, we find that these myomas usually get smaller. We have been able to carry a large number of women through pregnancy. We have Cesarean sections and have found that these myomas have undergone very marked hyaline and fatty degeneration. In some cases there have been palpable myomas which later have practically disappeared. Either there is a fundamental difference in the response of the human species from that of the guinea pig

or it may be that in the guinea pig the uterus is adrenergic and in the human cholinergic. I don't think that we should draw any clinical conclusions from experimental work on another species but we must recognize that the fibromyomas in the human may have a different pathogenesis, and that inferences may not apply to the human which apply to the guinea pig.

A. Segaloff: As Dr. Dobriner said yesterday, we have all been very concerned about the rate of absorption of these steroids. The rate of absorption from pellets such as employed by Dr. Lipschutz is probably the best index of the solubility of steroids in tissue fluids. I would appreciate it if Dr. Lipschutz would give us some idea of the relative rate of absorption of the pregnenolone compounds to which we have been referring.

A. Lipschutz: In reference to the comments of Dr. Gonzalez-Guzman, I feel indeed that a study of the cytologic events in experimental tumorigenesis would be of utmost importance. We have not done such studies. We must take into consideration that different types of tumors are due to quite different hormones. In our experiments the fibroid is due to the action of estrogens. On the contrary, the experimental tumors of the ovary are due to G.T., or gonadotrophic hormones. One must also be aware that no less than four different hypophyseal hormones are able to induce tumorous growth. We mentioned G.T., or gonadotrophic hormones, producing tumors of the ovary, and probably also of the testicle. We know from Wooley's work that C.T., or corticotrophic hormone, is also tumorigenic. In the work of the group of Bielschowsky, now in New Zealand, experimental evidence was obtained that T.T., thyrotrophic hormone, can cause tumors of the thyroid. And finally, H. M. Evans' group has produced evidence that S.T., the somatotrophic hormone, can produce tumors. It is well known from the work of Huggins that in the human body, testosterone is responsible for the tumorous growth of the prostate. Thus, there is sufficient evidence that many different hormones, steroid and nonsteroid, can produce tumoral growth. In cytological work, as implied by Dr. Gonzalez-Guzman, it would be necessary to distinguish among all these tumorigenic conditions.

As to the interesting remarks of Dr. Abarbanel, I may say the following: Certainly the fact that you can produce fibroids in the guinea pig with minute quantities of estrogen does by no means mean that fibroids in women are due to estrogen. It may be so, but we cannot provide proof. One cannot even produce these fibroids in anthropoids. One of my research students, now Professor Vargas, went to the laboratory of Dr. Corner to conduct similar experiments with *Macacus rhesus.* He was unable to reproduce these estrogen-induced abdominal tumors in this species. We have also used *Cebus apela,* a South American anthropoid; we were unable to produce abdominal fibroids even by treatment with estrogen for three years. Therefore, we don't know what happens in women during the initiation of fibroids. I suppose that estrogen is in

play; it is well known that in the menopause, uterine fibroids begin to shrink. The hormonal situation in women is certainly much more complicated than in our guinea pigs.

It might be well to recall the hereditary adenocarcinoma of the mammary gland in mice. We have known for some time that this is hereditary; it occurs with frequency in a given strain. Later, it became known from the work of Loeb and Lacassagne that estrogen is necessary for the development of this tumor in the respective strain. And finally, from the studies of Bittner, it became known that a virus also is necessary. Thus, we have no less than three different factors responsible for the origin of these tumors of the mammary gland. We think the fact that fibroids can be induced by estrogen is only part of the picture. In addition, most of our estrogen-induced fibroids are structurally different from those seen in women.

Another highly important statement should be emphasized: The antitumorigenic action of the steroids differs according to the species and according to the type of the tumor. This is fundamental and is one of the greatest difficulties of scientific work in this field. For instance, an abnormal or an atypical growth of the breast in humans can be counteracted in certain cases by androgens. In the guinea pig, progesterone, desoxycorticosterone, and pregnenolone do not counteract proliferation of the mammary gland. On the contrary, they enhance its growth. There is also in the mammary gland of the guinea pig, under the influence of estrogen, a metaplastic condition of the acini; this metaplastic condition also is enhanced by testosterone propionate. All this is quite different from what is known in humans.

I may mention at this time another interesting fact. One can induce with estrogen, as you all know, a typical proliferation of the anterior lobe of the hypophysis, particularly in rats and mice, but not in the guinea pig. One can counteract this hypophyseal proliferation with androgens, but not with progesterone; this again seems a conflicting fact since progesterone is the most powerful steroid in counteracting the growth of experimental fibroids.

Dr. Segaloff has asked whether I can give definite figures concerning the rate of absorption of different steroids from subcutaneously implanted pellets. One of my associates, Dr. Fuenzalida, has recently summarized our findings in his paper published in the November issue of the *Journal of Clinical Endocrinology*. I may add some data with respect to pregnenolones. Pregnenolone acetate was absorbed at a rate of 2.2 μg. per square millimeter of the pellet per day. To obtain an absorption of about 1000 μg. per day, 10 or more pellets have to be implanted. These figures suggest that it would not be possible to apply this method of administration to humans when an absorption of this quantity of pregnenolone is desired.

6

The Adrenal Cortex and Its Secretory Products

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This is a review of some recent studies on the nature of adrenal secretory activity and the possible mechanism of adrenal steroidogenesis. Chemical studies initiated in 1935 by Reichstein in Switzerland, and by Kendall, and by Wintersteiner and Pfiffner in the United States, led to the isolation and characterization, from adrenal gland extracts, of 28 crystalline steroids and a residual amorphous fraction.[1] Six of the crystalline compounds and the amorphous fraction proved to be physiologically active. Although these studies were of great value in clarifying the structure of the active principles of the adrenal cortex, they did not contribute to the problem of identifying the actual secretory products of this gland. The most direct approach to this problem is obviously to analyze the steroids released from intact adrenals, but until recently this has not been undertaken, owing to the lack of appropriate methods of analysis. The recent development of microanalytical methods for the isolation and identification of adrenocortical steroids[2-4] has made such a study possible. Two types of preparations have been employed; the removal of adrenal venous blood from living animals,[5] and the perfusion of isolated glands with homologous blood.[6] Experiments with the first type of preparation were carried out by the author in collaboration with Drs. Reich and Nelson[7] as follows: a cannula was inserted into the left adrenal vein of dogs receiving ACTH, and the blood was collected and extracted. Analysis of these extracts revealed the presence of 17-hydroxycorticosterone (Compound F) and corticosterone (Compound B). The second set of experiments, in which the technique of the perfusion of the isolated beef adrenal was used, were carried out by the author in collaboration with Drs. Hechter and Pincus. It was found that beef adrenals, perfused with homologous blood containing added ACTH, synthesize and release a variety of steroids into the perfusion medium. Up to 15 α-ketols were found, but again Compounds F and B were found to be the principal components of such adrenal perfusates.[8, 9] It appears, therefore, from the evidence at hand that Compounds F and B are the main secretory products of the adrenal cortex.

The second problem to be considered is the biosynthesis of the adrenal steroids. It has been suggested many times that cholesterol is perhaps the natural precursor of the adrenal hormones. One indirect piece of evidence often mentioned in support of this hypothesis is the observation that there is a pronounced fall in adrenal cholesterol following the injection of ACTH.[10-12] If this hypothesis is correct, then the adrenal cortex must be able to effect the necessary chemical changes to transform cholesterol into Compound F (Fig. 1). This transformation involves the following reactions: (1) oxidative cleavage of the side-chain of cholesterol yielding a two-carbon chain of the structure CH_3-CO-,

FIGURE 1

(2) oxidation of the $\triangle^5$-3β-hydroxyl function into a $\triangle^4$-3 keto structure, and (3) introduction of oxygen functions in the form of hydroxyl groups in positions 11, 17, and 21. The transformation product of cholesterol after reaction (1) is known as $\triangle^5$-pregnenolone (Fig. 1). Dr. Hechter and collaborators at Worcester have been able to demonstrate, using the perfusion technique, that $\triangle^5$-pregnenolone is indeed transformed by the adrenal gland into Compound F.[8] This is a very significant fact in view of the reported beneficial therapeutic effects of $\triangle^5$-pregnenolone in some cases of rheumatoid arthritis. It might well be that this effect of $\triangle^5$-pregnenolone is due to its transformation by the adrenal glands of the patient into active corticosteroids which are the actual effective agents. Some experiments are being carried out by the author to test the validity of this hypothesis.

Toward this end, some recent experiments carried out by the author in collaboration with Drs. Hechter and Pincus appear to furnish direct evidence in support of the view that cholesterol is a precursor of adrenocortical steroids.[13] Groups of five beef adrenal glands were perfused in parallel from a manifold for four hours with 1 liter of homologous citrated blood containing 25 mg. ACTH (Armour). The corticosteroids were extracted, and the extracts fractionated by paper chromatography.[2, 3] When 10 mg. of C^{14}-carboxyl labeled sodium acetate having a radioactivity of 5.8×10^6 c/m/m* were added to the blood at the initiation of the perfusion, Compounds F and B were isolated with radioactivities of 319 and 309 c/m/m respectively; in a second similar experiment, the c/m/m for each was 219 and 208, respectively. A similar perfusion of cholesterol labeled in position 3 with C^{14} prepared from radiocholestenone

TABLE I

THE SPECIFIC ACTIVITIES OF CORTICAL HORMONES AND THEIR DERIVATIVES ISOLATED FROM AN ADRENAL PERFUSION EXPERIMENT WITH $CH_3C^{14}OONa$

	mg.	*c/m/m*
1. Free Compound F	0.125	319
2. After rechromatography	0.115	340
3. Compound F acetate	0.130	362
4. Compound F propionate	0.098	332
1. Free Compound B	0.130	305
2. After rechromatography	0.090	326
3. Compound B acetate	0.123	294
4. Compound B propionate	0.110	342

by Drs. Schwenk, Gut, and Belisle, was conducted in which 90 mg. of radiocholesterol (300 c/m/m) was used. Compounds F and B were isolated, with activities of 25 and 18 c/m/m respectively. The data of Table I indicate that the radioactivity is a property of the compounds isolated since there was no significant alteration of the specific activity when the compounds were rechromatographed or when derivatives were prepared. These data indicate that both acetate and cholesterol can be transformed by the isolated adrenal into adrenocortical steroids. Since in adrenal slices, cholesterol has been shown to arise from acetate condensation,[14] it seems probable that cholesterol is an intermediate in the reactions leading to corticosteroid synthesis from acetate.

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* C/m/m—counts/millimol/minute.

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Discussion

There was no discussion following this presentation.

7

Comments on the Nonclinical Studies of the Steroid Symposium

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The purpose of this paper is to summarize briefly and comment upon the biological and physiological data dealing with animals which have been presented at this conference. Two points should be made immediately: (1) The time at my disposal for the assembling of these remarks has been very limited, since it was necessary for me first to hear the papers and discussions before this presentation could be assembled; and (2) the thoughts and recommendations for future research are my own opinions, and are not to be construed as either necessarily completely correct or as encompassing all of the worthwhile ideas and recommendations which should derive from this conference. I know that many members of the conference have their own evaluations of studies in the field of steroids, and I hope that these will be revealed before the end of our sessions. Also, I trust I am reporting correctly the major conclusions which have been reached in the papers presented.

The papers dealing with animal experiments, although relatively few in number, have provided much of both practical and theoretical interest. Relatively little time will be devoted to summarizing the papers which have been presented. Rather, the salient features of those presentations will be recalled, and the remainder of the time will be assigned to a certain degree of speculation and to suggestions for future investigations.

Dr. Selye listed what he termed eight independent actions of the various steroids; although the word "independent" was used, the discussion emphasized the previously known fact that there may be overlapping of steroidal actions. Nevertheless, the classification of steroids into folliculoid, testoid, luteoid, corticoid, spermatogenic, renotrophic, anesthetic, and antifibromatogenic types appears reasonable on the basis that one of these types of activities represents the chief biological manifestation of a particular steroid at a given dose level. However, the classification should not rigidify our thinking in connection with that steroid.

Dr. Selye pointed out that in his laboratory most steroids have been screened at very high dose levels. He stressed the importance of high doses in bringing out the physiological effects of certain steroids which might

have relatively weak biological potency. Of the 11-desoxy steroids studied, pregnenolone exhibited a marked degree of spermatogenic activity, being more striking in this regard than any other compound he had studied. It is of some interest that pregnenolone has been demonstrated to be a constituent of testicular tissue.

In experiments with desoxycortisone (Compound S), Dr. Selye reported that this substance produced the same morphological changes, in properly sensitized animals, as did desoxycorticosterone acetate, although seven times the dose of desoxycortisone, as compared to desoxycorticosterone acetate, was required. Finally, Dr. Selye presented evidence which he believes demonstrates that growth hormone, or somatotrophin, is identical with the factor he has studied in lyophilized anterior pituitary (LAP) extracts. This factor, given to experimental animals on a high sodium diet, produces a wide variety of histological lesions in rats. The effect of somatotrophin is evidenced only in animals given small amounts of ACTH to maintain their adrenal cortex at a properly secreting level. Somatotrophin may act synergistically with mineral corticoids at the peripheral tissues. Certain actions of somatotrophin are not mediated via the adrenals but may act directly on connective tissue to cause proliferation. Dr. Selye made the interesting suggestion that perhaps in diseases in which cortisone and ACTH are contraindicated, a combination of somatotrophin and desoxy steroids may have therapeutic benefit.

In the third paper of the conference, Dr. Nahum presented the results of a study of the effects of progesterone and pregnenolone upon the contractility and excitability of cardiac muscle, examined both in the isolated papillary cardiac muscle of the cat, and following intravenously administered steroids in the anesthetized, intact dog.

Dr. Nahum's data demonstrated that progesterone and pregnenolone had similar effects in that both decreased contractility and increased excitability and rhythmicity of cardiac muscle. Of significant interest was the demonstration that propylene glycol, used as a solvent for the steroid hormones, has in itself marked effects on cardiac muscle.

In the comparison of the effects of pregnenolone and progesterone with digitalis, it was pointed out that these steroids resembled digitalis in their effects on excitability and rhythmicity, and have an opposite effect on contractility.

In the fourth paper of the conference, Dr. Stock reported on some of the data obtained at the Sloan-Kettering Institute in the screening of various steroids with respect to their effect on normal and abnormal growth in experimental animals. Using chick embryo growth as a screening method, it was shown that the most striking inhibitory effects on growth were obtained with Compound F and with cortisone. Compound A acetate had a lesser effect in inhibiting the growth of the chick embryo; 21-desoxycortisone had a similar degree of activity in the chick embryo.

The second type of screening study was done with various experimental tumors in animals, with particular emphasis being placed on the data obtained with the transplantable mouse lymphosarcoma. With the

latter tumor, Compound E showed the most striking activity, Compound A had one-third to one-fifth the activity of Compound E, and Compound S had approximately one-twentieth the activity of Compound E. These effects were seen in animals in which the steroid was given for seven days, commencing 24 hours after implantation of the malignant tissue.

Of considerable interest was Dr. Stock's report of Dr. Money's observation that, following the injection of Compound L in doses of 15 or 30 mg. daily for several weeks in rats, there was an increase in adrenal weight, a decrease in thymus weight, and an increase in weight of lymph nodes. In similarly treated animals, Dr. Bodansky in the same laboratory found that there was a decrease in the plasma vitamin A level and an increase in liver vitamin A concentration. This study of vitamin A suggests an additional new index or measurement, not applied previously, for assessing possible biological potency of a steroid.

An additional type of steroid screening program, with particular reference to growth inhibiting effects in experimental animals, has been presented by Dr. Lipschutz and his associates. The test object employed was the estrogen-induced abdominal tumor, which can be produced consistently in guinea pigs. This experimental study of what has been designated the antifibromatogenic action of steroids, suggests the following conclusions:

1. The antifibromatogenic activity appears to be dependent upon the presence of a ketonic group in position 3 of the steroid nucleus, although not all 3-keto compounds are antifibromatogenic.

2. Of the steroids tested in these studies, progesterone has been shown to be the most potent.

3. The antifibromatogenic action of the steroids appears to be dissociated from, that is, not related to, the other physiological activities of these steroids, e.g. progestational, corticoid, or virilizing actions. The data suggest, therefore, that the antifibromatogenic activity is an independent property of certain steroids.

4. There is apparently no essential correlation between the antitumorigenic activity of a steroid on a tissue in one species and its predictable behavior on the same tissue in another species. This is illustrated perhaps most strikingly by the differential behavior of testosterone in cancer of the breast in humans as compared to its ability to stimulate abnormal mammary proliferation in guinea pigs.

5. The route of administration and rate of absorption of the steroid being tested is of primary importance; most effective antitumorigenic activity is manifested when absorption is continuous, i.e. as a result of subcutaneous implantation of pellets.

6. A 3-hydroxy steroid, e.g. pregnenolone, may have antifibromatogenic activity, but the dose required appears to be many times that of the 3-keto compounds, e.g. progesterone.

At this point it would appear worthwhile to comment briefly on the above four presentations from the point of view of (1) relating the findings to the subject of the conference, namely, Steroids in Experimental

and Clinical Practice, with particular reference to the 11-desoxy steroids and (2) indicating some of the variables which appear to be of obvious importance as a result of the experimental findings.

It seems clear that:

1. A given steroid may have several types of physiological activities, with these activities being manifest in varying degrees.

2. Certain 11-desoxy steroids, namely, progesterone, pregnenolone, Compound S, and Compound L have been shown to have physiological effects in experimental animals.

3. The data may suggest that, as in the case of the several steroids with vitamin D-like activity and in the three naturally occurring estrogens, there is possibly a broad spectrum of steroids; spectrum in the sense both of slight to extensive alterations in chemical structure and of slight to extensive variations in physiological effects. It would appear that there should be a broad and fundamental program designed to correlate chemical structure with physiological and pharmacological responses. From this program should come much of both theoretical and practical importance.

With respect to the variables which have evolved from the animal experiments under consideration, it seems quite clear that these are many in number. One of the most important variables in these studies is the question of absorption. This will be referred to again later. One may list secondly the animal species, since the same steroid in the same dose does not necessarily produce the same effect when studied in two different animal species. A third important variable is the dose in which a particular steroid is administered. It should be pointed out particularly that steroids, like other physiologically active agents, might produce quite divergent and even opposite results when studied in relatively low, as compared with high, dose ranges. This point is mentioned as a result of Dr. Selye's paper in which, as he stressed, his laboratory has screened steroids at high dose levels. It is true that the use of high doses may sometimes reveal physiological effects or activities which are not seen with lower doses because the particular steroid may be of relatively low potency in the particular test being applied. Nevertheless, it cannot be emphasized too strongly that the action produced by a steroid in large dosages is not necessarily the response which would be obtained with the steroid in smaller doses. Indeed, in smaller quantities, a compound might produce quite an opposite effect. There are many examples to substantiate the important relationship between dose and response. We have seen in recent years that Compound E in small doses may cause sodium retention, whereas in larger amounts it may cause sodium excretion. A classical example from the field of pharmacology is the stimulating or exciting action, on the one hand, of small doses of morphine, and the depressing action, on the other, of larger doses of this compound.

Another variable in experiments with steroids, and one of very great importance, is the problem of the food intake of the experimental animals. Many of the steroids, particularly when used in large doses, have been

described as causing inhibition of body weight. Dr. Stock indicated that his group has recognized that many of their steroids tested caused a reduction in food intake, which in itself retards weight increments. Furthermore, a reduced caloric intake is a potent factor in the production of endocrine imbalances, notably a reduction in the elaboration of the pituitary gonadotrophins, as well as a tendency to augment endogenous production of ACTH. In our laboratory, for example, Dr. Adams[1] has demonstrated that restricted caloric intake could in itself produce marked retardation of the growth of an otherwise rapidly progressing lymphosarcoma in the mouse. The whole problem of tissue response in circumstances of suboptimal nutrition is one of prime importance in screening programs of steroids. Interesting examples are the interrelationships seen by Hertz between the level of dietary folic acid and the response to steroids, and the studies of Gassner on vitamin B_{12} and steroid action.

Finally, in connection with obvious experimental variables, a point should be made concerning the route and rate of administration of a particular compound being tested. It is a *sine qua non* of physiological studies that the rate and degree of response of a particular tissue to an administered compound is dependent upon the concentration level of the stimulating agent which has been established at the cells of the particular tissue or organ under study. The route of administration of a compound and the rate of its absorption from the site of injection are the controlling factors in this connection. One has seen this point emphasized in the work of Dr. Lipschutz and associates with steroid pellets. Dr. Wooley has demonstrated recently the relative ineffectiveness of ACTH in affecting the growth of lymphoid tumors as compared to the effectiveness of cortisone in this respect. The fact that this was due chiefly to the variable under discussion, i.e. the route of administration and dose, was demonstrated by data showing that when ACTH, soluble in water and therefore absorbed rapidly, was given at exceedingly frequent intervals in order to maintain a significant amount of the substance in the circulation, a growth inhibitory effect on lymphoid tumors could be demonstrated.

Finally, in this discussion of experimental variables, attention may be directed to another possible variable which, while it may be difficult to control, is of fundamental importance in the interpretation of experimental data. Reference is made to what may perhaps be termed the multipotentiality of cells. It is generally recognized, and its significance should not be underestimated, that the endogenous production of a particular steroid is not confined to a particular tissue or organ but may be elaborated at more than one site in the organism. We are at the present time almost wholly unaware of whether the relative importance of two different tissues in the production of a specific compound may alter as a consequence of the administration of an exogenous steroid. Moreover, we recognize that under certain experimental conditions, as a result of an accentuated need of the organism for a given compound, a tissue not concerned ordinarily with the elaboration of this substance may initiate or

increase its production. A well-known example of this is the formation of extra-adrenocortical cells in rats adrenalectomized in two stages, and, of course, the augmented production of androgens by the adrenals of castrate animals. In this connection, one recalls the striking experiments that Dr. Dorfman and his associates[2] have reported on castrate-adrenalectomized monkeys. In those experiments, castration of the male monkey produced a fall in ketosteroid excretion. After a period of time, the ketosteroid excretion began to rise and tended to approach the preoperative level. This, of course, is the classical increased production of androgenic substances by the adrenal following castration. However, when the adrenals were also removed from these castrate animals, although the ketosteroid excretion dropped to a low level, there were still present in the urine ketosteroids in amounts approximately 10 per cent of the preadrenalectomy level. These excretory products were present in the ketonic fraction. Furthermore, these urines from castrate-adrenalectomized monkeys continued to exhibit an androgenic titer approximately 10 per cent that of the preoperative level. What is the physiological source of these steroids, and how does exogenous steroid administration affect their production? Are steroids produced and metabolized only in tissues which have been examined because of both logical and traditional thinking?

The final nonclinical paper to be commented upon is considered apart from the others since the experimental objectives and procedures were quite different from those of the other four papers which have been discussed. This is the paper presented by Dr. Zaffaroni. These experiments had as their objective an answer to the very important question: What are the true secretory products of the adrenal cortex? The work was also designed to examine the possible capacity of the adrenal cortex to conduct transformations and interconversions among steroids.

It was reported by Dr. Zaffaroni that although perfusion of the isolated adrenal gland with ACTH resulted in the production of some 12 to 14 different steroids, all but two of these are produced in relatively minute quantities. The steroids secreted in large quantity by the adrenal appear to be Compound F and corticosterone. These observations are of much interest in the general field of adrenal physiology and clinical practice, because the emphasis to date has been largely on cortisone and on how much cortisone the gland can produce. It seems quite clear that relatively little cortisone is produced by the adrenal under normal conditions of stimulation by ACTH, but rather that the more important steroids manufactured are the 11-hydroxy compounds. It is interesting, in this connection, to recall that in the chemical studies of Reichstein and his colleagues, the steroids isolated from adrenal glands were, for the most part, 11-hydroxy compounds, while those isolated by Kendall were characterized to a much greater degree as being 11-keto compounds. One wonders to what extent this difference in data might be related to the relative period of time elapsing following removal of the glands at the slaughter house and their actual chemical extraction and study.

The observation of Dr. Zaffaroni and his colleagues that only two

steroids are produced in significant amount on stimulation of the adrenal gland *in vitro* also leads to the question of whether the abnormal urinary steroid pattern in various diseases is evidence of altered synthetic mechanisms in the adrenal with production of steroids which could be etiologic factors in disease, or whether these urinary steroid patterns are a result of differences in the way diseased tissues in the body handle or transform the two primary steroids produced by the adrenal cortex? Also, could the numerous other steroids produced in minute amounts by the adrenal cortex under normal circumstances be secreted in pathologically significant quantity, leading to the appearance of disease?

In this connection, if possible, it would be of considerable interest to study perfusates of adrenals from either experimental or clinical subjects with one or more diseased states. Even though it is not possible experimentally to reproduce some of the interesting human diseases in animals, one could nevertheless begin this program in animals with such experimentally altered conditions as pregnancy and other endocrine imbalances.

Finally, in connection with Dr. Zaffaroni's paper there will be recalled his demonstration of the conversion of cholesterol to Compound F and corticosterone by the adrenal gland *in vitro*, as well as the conversion of carbon 14-labeled acetate to these two compounds during adrenal perfusion. Additional evidence was presented that Δ^5-pregnenolone is converted to Compound F during adrenal perfusion. The possible role of this 11-desoxy steroid as an intermediate in the transformation of cholesterol to naturally occurring adrenal corticosteroids appears to be a logical segment of hypotheses regarding the *in vivo* synthesis of adrenal steroids.

We come now to that portion of this presentation devoted to suggestions or recommendations for future research. It seemed that in approaching this topic at the present time, a practical starting point for suggestions and problems would be a consideration of the factors which could have etiologic significance in diseases in which steroids might be concerned, either as causative or therapeutic agents. From these considerations, one could pass logically to the problems arising from these factors, and then perhaps to a consideration of the methods of attacking these problems, although this latter point will be touched upon only briefly, since a statement of the problem will suggest to the audience and the reader the several obvious methods of attack.

In considering the data obtained in various diseases which have been reported to be ameliorated in part by ACTH, cortisone, Compound F and, in some instances, certain of the 11-desoxy steroids, the evidence suggests strongly that in individuals with one or another of these diseases there is little evidence of impaired pituitary-adrenal cortical responsivity. That is, such individuals, when given a nonspecific stimulus known to activate pituitary-adrenal cortical secretion, e.g. epinephrine or histamine, give evidence of a good release of their own pituitary ACTH. Therefore, the pituitary end of the pituitary-adrenal cortical axis appears to be responsive. Secondly, such individuals given exogenous ACTH appear to have good physiological, and in a significant number of instances, thera-

peutic responses. Therefore, their own adrenals are capable of elaborating therapeutically active steroids. Since in these diseased states these individuals should be under the influence of a variety of stress stimuli accompanying the pathology of the diseased state, the question arises as to why their own pituitary-adrenal cortical mechanism, in the face of the stimulating stress of disease, does not elaborate the additional therapeutically needed adrenocortical steroids, and why relatively large doses of these steroids have to be given initially to produce a significant remission? This state of affairs could exist as a result of one or more of the following theoretical possibilities:

1. Although there is evidence, cited above, for pituitary adrenocortical responses in these individuals, this may be only an acute, short-term phenomenon and there may be a failure of continuing production of the steroids needed.

2. The rate of synthesis of needed steroids by the adrenal cortex may be at a normal or even above normal level, but this production is inadequate for peripheral tissue needs. This inadequate production could be due to deficiency in supply of raw materials or a deficiency in concentration of one or more of the enzyme systems concerned with the steroid synthesizing processes.

3. As a consequence of the stress of disease, there could be an accelerated production of needed steroids, but the rate of their utilization by the tissues may be enormously exaggerated and therefore the steroid supply, even though perhaps greater than normal, is inadequate.

4. There may be a block at the tissue level in the utilization of these steroids, including perhaps a decrease in the peripheral tissue response to a dose of steroid, which, in the normal individual, would induce a characteristic physiological reaction.

5. There may be a derangement or an aberration in the normal picture of steroid synthesizing mechanisms of the adrenal cortex, with the result that a non-normally produced steroid is manufactured. This compound, or perhaps compounds, if causing undesirable physiological effects, would become an etiologic factor in a disease syndrome.

6. Diseased tissues may exhibit aberrations in their normal steroid metabolic picture and alter the few naturally produced physiologic steroids into pathologic substances which have deleterious effects.

7. Finally, a pathological process may ensue in a tissue or organ as a consequence of the fact that the normal capacity of the cells to metabolize or deal with a normally occurring steroid is inadequate. Under these circumstances a steroid which in moderate doses has beneficial effects, may, when its concentration builds up in a particular cell as a result of nonutilization at a normal rate, produce deleterious effects.

The problems which arise from these considerations, as well as from the presentations and discussions of this conference, particularly in relation to the animal experiments which have been presented, are many in number. They may be indicated briefly as follows:

1. It is desirable to test or screen a wide variety of steroids in animals,

using as many chemical and physiological indices as possible. We have seen that certain 11-desoxy steroids have physiological effects, and since their production in the laboratory at the present time is accomplished more readily than that of the 11-oxy steroids, the former compounds should be examined extensively. In this connection, one should keep in mind that 11-desoxy steroids, with relatively low activity, should also be examined in conjunction with 11-oxy steroids for a possible synergistic effect. In this animal testing or screening program, due recognition should be given to the age, sex, and species of animal as well as to dietary intake. The program should include (a) evaluation of toxicity, (b) examination of various dose levels in order to correlate response to dose, and (c) an attempt to correlate structure with physiological response. Not only the established indices of responses should be employed but new indices should be sought. We have seen from Dr. Nahum's study that an additional experimental index is available, i.e. response of cardiac muscle. His experiments, which were done at only one dose level, should be extended to other dose levels as well as to other steroids.

In connection with the animal studies, it is the author's opinion that a valuable program of studying steroids would be the examination of the effects of these compounds in animals maintained on "sensitizing" (high sodium) diets and given desoxycorticosterone acetate. No claim is being made that the experimental arthritic-like states produced under these conditions are identical with human disease. However, this experimental subject, as well as the guinea pig on a low vitamin C intake, should be studied carefully with the view of evolving a possible test animal for assessing a possible aspect of steroid function.

2. One of the outstanding problems in the steroid field, both with respect to animal and human investigation, is the problem of solubility of these compounds. This constitutes a real challenge to the organic and physical chemists. In the case of aqueous suspensions the problem of most effective particle size should be investigated. The organic chemist may be able to provide us with chemical derivatives of steroids which have a greater degree of solubility than the parent compound.

3. A third problem has some relationship to the second, since the solubility of a compound regulates significantly its absorption into the organism, whether that absorption is from the gastrointestinal tract or from a site of parenteral injection. In connection with oral medication, practically nothing is known regarding either the rate or the degree of absorption of individual steroids. All of the evidence for or against absorption from the gut has been based on therapeutic and biological responses. This fundamental problem of absorption of steroids after oral administration can be studied extensively in a quantitative manner in experimental animals.

4. The overall metabolic fate of various steroids needs examination. With the availability of the methods of paper chromatography, infrared spectroscopy, and fluorimetry, the metabolic excretory products of steroids can now be studied in experimental animals as well as in the human.

5. The above metabolic fate aspect of the steroid field is related, of course, to transformations in the steroid nucleus which may occur in the various tissues of the body. This can be studied by perfusion techniques and by tissue incubation experiments. The tissue incubation studies are also an *in vitro* method for studying the rate and extent of utilization of various steroids, and might answer the question of whether a diseased tissue, taken by biopsy, does have a diminished or accelerated rate of utilization of a particular steroid. This approach, of course, also is an aspect of the fundamentally important field of elucidating the enzyme systems concerned with the metabolism of steroids. The capacity of tissues to hydroxylate an 11-desoxy steroid at the 11-position is an established experimental fact on the basis of the work of Dorfman, Zaffaroni, Samuels and Nelson, and Hechter, Pincus and their associates. Moreover, of prime importance in the enzymatic studies is the fact that any degree of elucidation of the basic processes affected by these steroids at the cellular level should give enormous impetus to the synthetic program by indicating the important functional groupings in the steroid nucleus, as well as possibly in related compounds.

6. In connection with the capacity of tissues to transform 11-desoxy steroids into 11-hydroxy substances, the question arises as to whether the rate of this process is diminished in the hypothyroid animal, or perhaps of greater significance, whether the rate and extent of this physiological 11-hydroxylation can be augmented by elevating tissue reactivity to the hyperthyroid stage.

In recapitulation, therefore, it may be suggested that we are on the threshold of what might be termed a "steroid era." The organic chemist has and will continue to produce an increasing number of new steroids, each of which may be examined in detail physiologically. An intensive effort is indicated to determine whether the great diversity of profound physiological alterations effected by normally produced Compound F and corticosterone cannot be mimicked at least in part or perhaps wholly by structurally related substances. The history of therapeutic agents is replete with examples of structurally related compounds which have either identical or related pharmacological or physiological effects, and with studies in which the organic chemist has been able to improve on the potency and activity of naturally occurring substances by the production of synthetically related compounds. In many instances, by alteration of structure, it has been possible to retain certain desirable physiological responses in a therapeutic agent with concomitant suppression of undesirable side-effects.

In closing, opportunity is taken to recapitulate certain additional facts derived from this conference as viewed and assessed from listening to the presentations and discussions.

One of the most important of the unsolved problems of the conference is that of the absorption of pregnenolone, either from the gut or from the site of parenteral injection. The lack of an answer to this problem has beclouded much of the discussion. Is it possible that some of the conflict-

ing data are attributable to differences in absorption of this compound from individual to individual? Certainly it is known, particularly when substances are given orally, that patients exhibit varying capacities of absorption. Could it be that some of the differences in effects reported with pregnenolone represent instances in which the compound was absorbed to a varying degree? The absorption factor would explain the failure to demonstrate striking metabolic alterations following pregnenolone administration. That pregnenolone does have a metabolic influence when it is absorbed in sufficient quantity would seem to be clear from the demonstration that when it is placed in blood in contact with cardiac muscle *in vitro*, and when it is injected intravenously, pregnenolone does indeed have a profound metabolic effect. Moreover, Dr. Selye has indicated an effect of injected pregnenolone on spermatogenesis. It will be recalled that Dr. Selye's doses were large, and even a low degree of absorbability could have resulted in the entrance of sufficient pregnenolone into the circulation to produce the observed effects. Is it not significant that Dr. Lipschutz obtained an antifibromatogenic action with pregnenolone pellets, which is a technique of administration that provides maximum opportunity for absorption?

Conflicting data can result only from either uncontrolled or unrecognized experimental variables. The challenge in the field of the physiological and clinical significance of steroids lies in eliminating or standardizing these variables so that existing and new steroids can be assessed with scientific accuracy.

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Discussion

There was no discussion following this presentation.

8

Adrenal Function in Subjects Receiving Cortisone and Pregnenolone Therapy

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The data presented in this paper represent the initial steps of an attempt to assess adrenocortical function in patients previous to and during a period of steroid therapy. The objective of this attempt is a fairly simple one: To determine whether or not the therapeutic effects of a given steroid may be related to the adrenocortical activity of the subjects receiving it. In order to elucidate the nature of adrenal function, two principal analytical approaches have been used: (1) the measurement of response to an Exton-Rose glucose tolerance test and to the administration of adrenocorticotrophic hormone (ACTH), and (2) an analysis of the excretion of urinary corticosteroids.

Subjects and Methods

The subjects employed in these studies were men and women with an unequivocal diagnosis of rheumatoid arthritis, and schizophrenic men. During the week preceding the initiation of steroid administration each of the arthritic subjects was given the two-dose Exton-Rose glucose tolerance test on one day and on the succeeding day given an amount of ACTH equivalent to 25 mg. of Armour Standard LA-1A.[1] The schizophrenic men were given only one ACTH test during the premedication week, but from all subjects, during a test-free period, a 48-hour urine specimen was collected for corticosteroid extraction and analysis. At the end of a week, at a time when steroid therapy was being continued, these procedures were repeated.

Urine and blood specimens were collected at the beginning of each test. These are the "pretest" samples. The urine specimens were analyzed for creatinine, uric acid, inorganic phosphate, potassium, sodium, and 17-ketosteroid content, and the blood specimens for lymphocyte, eosinophil, and cholesterol content using methods previously described.[1-3] At ½, 1, and 3 hours following the initiation of the glucose tolerance test, blood samples were taken, and at 3 hours a urine specimen was taken covering the total test period. In the ACTH test the blood specimens were

taken at 0, ½, 2, and 4 hours, and the test specimen of urine was taken at 4 hours.

The 48-hour urine specimens collected were subjected to continuous extraction by methylene chloride at pH 1 and the neutral lipids of the extract were assayed for reducing lipids (RL) by the Heard-Sobel molybdate method[4] and for formaldehydogenic steroid (FS).[5] The neutral lipids were separated into ketonic and nonketonic fractions and chromatographed on silica gel by methods previously described.[6] The ten fractions eluted from the silica gel columns were assayed for reducing lipids and for formaldehydogenic steroid. Of these fractions, three (nos. 2, 3, and 4) contained substances having roughly the polarity of 11-desoxycorticosterone, two (nos. 6 and 7) contained more polar substances (such as corticosterone, cortisone, etc.), and two (nos. 9 and 10) contained the most highly polar substances.

The data of this paper represent metabolic studies of (1) nine arthritic subjects (six women and three men) who received 500 mg. per day of pregnenolone acetate by mouth during the therapy period, (2) 12 arthritic subjects (six women and six men) who received oral cortisone acetate (300 mg. on the first day, 200 mg. on the second and third days, and 100 mg. daily thereafter), and (3) 12 schizophrenic men who received 100 mg. per day of cortisone acetate by intramuscular injection. All subjects underwent the tests described, but in certain instances, complete determination of all the biochemical variables was not possible. In the oral therapy series, placebo tablets (of lactose) were given during the premedication period; the schizophrenic men received no placebo injections.

Results

Basal Values. The mean basal values for each variable measured in the adrenal function tests during the premedication and medication periods are presented in Fig. 1. The urinary values are calculated as output per gram of creatinine. Thus in the group of arthritic patients who received oral cortisone acetate, the mean 17-ketosteroid output was 2.63 mg. per gram of creatinine in the premedication period and 3.29 mg. per gram of creatinine during the early part of the second week of medication, an increase of approximately 25 per cent for the medication series. Since each subject underwent two tests in each period, each mean value represents 24 determinations, two for each of the 12 patients. Similarly the group of subjects on pregnenolone therapy initially had a mean 17-ketosteroid output of 3.10 mg. per gram of creatinine and this fell to 2.19 mg. during the medication period, a drop of approximately 29 per cent. The schizophrenic men receiving cortisone intramuscularly had an initial output of 2.27 mg. which declined to 1.47 mg., a drop of 35 per cent. The basal output of 17-ketosteroids, therefore, increased during the medication period in the group receiving oral cortisone, and decreased in the pregnenolone series and in the schizophrenic group receiving cortisone by injection. Interpretation of these data requires caution: The pregnenolone effect appears to involve the 17-ketosteroid sparing action

FIG. 1. A comparison of the concentrations of 17-ketosteroids (17-Ks), potassium (K), sodium (Na), uric acid (U.A.), and inorganic phosphate (PO_4) in the urine, and of the eosinophils (EOS.), lymphocytes (LYMPHS.), and cholesterol (CHOLEST.) in the blood in three groups of patients preceding and during periods of medication with cortisone (CORT.) and pregnenolone (PREG.). Data for the arthritic patients are shown by the letter A and for schizophrenic patients by Ps.

which we previously noted in aviator subjects;[7] the inhibitory effect in the schizophrenic patients may involve the depression of endogenous production of 17-ketosteroid precursors, due, apparently to the inhibition of endogenous pituitary ACTH;[8] but the increased output in the arthritic patients receiving cortisone is not so easily explained. We suggest that the most likely explanation may inhere in the passage of the absorbed cortisone acetate through the liver with consequent production (by oxida-

tion of the side-chain) of a sufficient amount of 17-ketosteroid catabolites of cortisone to outweigh endogenous inhibition. Alternatively one would have to postulate stimulation of endogenous ACTH by cortisone or a metabolite thereof, or postulate that oral cortisone is not, like injected cortisone, ACTH-inhibiting so that endogenous 17-ketosteroid production is not interfered with and the 17-ketosteroid catabolites of cortisone are merely added to the basal endogenous output.

The basal potassium excretion in each group was reduced during the medication period by 24 per cent, 18 per cent, and 43 per cent, respectively. The sodium output was increased in the oral cortisone group by 19 per cent and decreased in the other two groups by 26 per cent and 32 per cent, respectively. Again, the net sodium-retaining effect of cortisone in the schizophrenic men was an expected result, and the pregnenolone effect may be due to the conversion of this substance to a steroid having sodium-retaining effects, but the sodium-excreting effect of oral cortisone is less easy to explain; again the conversion of cortisone by the liver to a sodium excretion inducer is suggested. Of course these electrolyte excretion changes may be merely accidental and reflective only of the dietary intake of sodium and potassium. The indications of a net retention of the latter in all groups is problematical, but the rebound from an initial increased output may be involved. In the case of the schizophrenic subjects, basal measurements were made on the first day of cortisone administration and a mean rise of 18 per cent in output over the premedication level was observed.

Basal uric acid output figures show little change in the arthritic subjects during the medication period, but the clear output increase of 20 per cent in the schizophrenic men is the expected result of cortisone administration. We might deduce that oral cortisone is converted by the liver to a substance which induces no uric acid excretion; similar ineffectiveness in this respect of a possible pregnenolone conversion product is suggested. The mean phosphate excretion increase observed in the schizophrenic subjects is a characteristic effect; we previously noted this response to adrenocortical extract administration in an acute test.[1, 2] No marked differences in the urinary levels occurred in the arthritic patients during medication, but the net decline in the pregnenolone group *may* indicate better carbohydrate utilization (as may the accompanying decline in potassium output).

The changes in concentration of blood eosinophils are most interesting. Again we observed an expected marked eosinopenia in the schizophrenic subjects, but in the oral cortisone series this did not occur. The slight decline (9 per cent) in the pregnenolone series is not significant. In all three groups there was an increase in the number of circulating lymphocytes, most marked in the oral cortisone series (39 per cent increase) and least (5 per cent increase) in the pregnenolone group. Here we observed a "typical" effect of cortisone therapy, the lymphocytosis following an initial lymphocytopenia. In the schizophrenic men a decline of about 10 per cent in mean blood lymphocyte concentration was, in

fact, observed on the first day of cortisone injection. The blood cholesterol changes were not notable in oral cortisone groups, but there was a tendency for blood cholesterol to increase during medication, whereas the decline of 13 per cent in the pregnenolone series represents a contrasting tendency.

The overall indications of these data are that after a week of medication (1) oral cortisone does not act metabolically like injected cortisone and (2) that pregnenolone in turn differs from both. As a general explanation we might suggest that cortisone is converted in the liver to a substance which is a 17-ketosteroid precursor, slightly salt-excreting and potassium-retaining in effect, not markedly effective on uric acid or inorganic phosphate excretion, non-eosinopenic, lymphocytotic, and, perhaps, slightly hypercholesterolemic. Oral pregnenolone, on the other hand, may be

FIG. 2. The course of the Exton-Rose glucose tolerance test in arthritic subjects before and during medication. *Circles,* before pregnenolone; *squares,* during pregnenolone; *inverted triangles,* before cortisone, *triangles,* during cortisone.

converted *in vivo* to a substance which is ketosteroid-sparing, salt- and potassium-retaining, not markedly effective on uric acid and inorganic phosphate excretion, slightly eosinopenic and lymphocytotic, and, perhaps, hypocholesterolemic. Such speculation must obviously be taken tentatively; control subjects and schizophrenic men exhibit characteristic effects of parenteral cortisone described for other arthritic subjects,[8] but the latter may be considered not "normal" even though previous studies[1, 2] show that their response to adrenocortical extract is entirely comparable to that of healthy, nonpsychotic men.

Glucose Tolerance Test. In Fig. 2 we present the mean blood sugar values of the arthritic subjects given the glucose tolerance tests. The premedication and medication tolerance curves are quite similar, but there is a slight suggestion of decreased tolerance in the subjects receiving oral cortisone. This is certainly so much less marked than one might expect

that again we must conclude that oral cortisone at one week after administration lacks much "diabetogenic" action.

In Fig. 3 we present the changes in the various indices measured in the course of the glucose tolerance tests. In calculating these data the basal, or pretest, values were taken as 100, and the test values were expressed as a percentage of the pretest value. In the case of the blood cell measurements, the mean of the various test values was taken. For comparison with the series on the arthritic subjects, we plotted in the upper graph similar tolerance test values obtained from a large group

FIG. 3. The percentage changes occurring in various urinary and blood constituents during the test period in subjects undergoing the Exton-Rose glucose tolerance test. The abbreviations are as in Fig. 1. The upper graph represents data for a series of normal male subjects. The total response index (TRI) is calculated as explained in the text.

of normal, healthy men. In the premedication period neither group exhibited any very marked response to the glucose administration. Thus the 17-ketosteroid output did not change significantly (changes +6.5 per cent and —4.0 per cent in the cortisone and pregnenolone groups respectively, compared with +35.5 per cent in the normal men). The characteristic potassium retention was observed, but the expected sodium excretion occurred only in the cortisone group. The expected normal mild phosphate retention did not occur, and the marked uric aciduria of normal subjects was greatly damped. The eosinophil response in the pregnenolone group was of the normal order of magnitude (but not in the cortisone group) and the expected lymphocytopenia was not evidenced. During the medication period in both groups, no very marked alteration of degree of response occurred, except for a more typical 17-ketosteroiduria and blood cell response in the cortisone series; on the other hand, the sodium and uric acid excretions shifted toward a less responsive level.

Fig. 3 shows the mean total response index (TRI) calculated from these data. The index is the mean of a number obtained by adding the percentage increases over pretest values of urinary 17-ketosteroids, sodium and uric acid, the percentage decrease in eosinophils, and twice the percentage decrease in blood lymphocytes. In the case of normal, healthy men (upper chart) this value was 29.4; the premedication values for the cortisone and pregnenolone groups were 12.0 and −1.4, respectively. Following medication there was clearly no significant change in either group. It may be concluded that the glucose tolerance test evokes diminished adrenocortical response in arthritic subjects. It is interesting to note that in a group of 38 schizophrenic men, the mean total response index was 5.7, compared to a control group value of 22.1.[3] In this latter case, it was suggested that the diminished response of the schizophrenic men could be attributed either to a failure to stimulate normal ACTH release or to a failure of the adrenal cortex to respond to a normal ACTH secretory release. A test of these alternatives in arthritic subjects is offered by the administration of ACTH. It is generally recognized that patients with rheumatoid arthritis are responsive to ACTH. Nonetheless, we undertook the 25 mg. ACTH test with these same subjects.

The ACTH Test. The magnitude of response of the arthritic subjects to ACTH during the premedication period was fairly characteristic, as shown by the data of Fig. 4. Increases in urinary 17-ketosteroids, potassium, and sodium during the test period were fairly normal, but there was a rather small uric acid excretion increase. Inorganic phosphate excretion was elevated, much like the result observed in ACTH tests with older men.[9] Eosinopenia occurred as expected, but there was no lymphocytopenia. The premedication total response index was identical in the two groups of arthritic patients, approximately +26 (in constructing the total response index in the ACTH tests, the percentage of change in potassium excretion is included also). The value of +26 is lower than the values obtained for two groups of normal men[9] previously examined (+47 for a younger age group and +32 for an older age group) so that a somewhat diminished quantitative response to this test is suggested. Oral cortisone and pregnenolone administration appear, by and large, to increase the degree of response of the arthritic subjects to ACTH. This was consistently evident in the ketosteroid and uric acid responses and irregularly evident in other responses. As a result, the total response index increased in both groups, to +36 in the pregnenolone series and to +39 in the cortisone series. In contrast, the schizophrenic men receiving intramuscular injections of cortisone exhibited a reduced degree of response during cortisone administration. This was evident in practically every index but was most obvious in the 17-ketosteroid, potassium, and uric acid responses. The total response index before medication was +28.9 and during cortisone, was +16.8.

The implication of the ACTH-response changes is that injected cortisone, presumably by its inhibitory effect on endogenous ACTH release, reduces the magnitude of adrenal response to ACTH whereas oral corti-

sone and pregnenolone do not exert the inhibitory effect. The observed improved degree of ACTH response during medication may be due either to a toning-up of the pituitary-adrenal mechanism or to an improvement of the magnitude of end-organ response.

Corticosteroid Excretion Studies. In Table I are presented the data in four groups of subjects on urinary corticosteroid determinations of the total neutral lipid before fractionation. In addition to the groups, dis-

FIG. 4. The percentage changes in the various urinary and blood constituents occurring during the test period following the administration of ACTH. The abbreviations are as in Fig. 1. Comparison of the changes and of the total response index (TRI) is made for the premedication and medication period.

cussed previously, the table includes data on two schizophrenic men who received 1 gm. per day of pregnenolone acetate by mouth. The data of the table demonstrate (1) an increase in both reducing lipids and formaldehydogenic substance in the arthritic subjects receiving cortisone, (2) an increase only in formaldehydogenic substance in the schizophrenic men receiving cortisone, and (3) small output changes in the arthritic and schizophrenic subjects receiving pregnenolone, the largest being the increase in reducing lipids in the schizophrenic men. These data indicate

TABLE I

MEAN CONCENTRATIONS OF URINARY CORTICOSTEROIDS FROM ARTHRITIC AND SCHIZOPHRENIC PATIENTS PRECEDING AND DURING MEDICATION. CONCENTRATION EXPRESSED AS MILLIGRAMS 11-DESOXYCORTICOSTERONE EQUIVALENT PER GRAM OF URINARY CREATININE

Subjects	*Medication*	*Number*	*Total Neutral Reducing Lipid (RL)*	*% Change*	*Total Neutral Formaldehydogenic Substance (FS)*	*% Change*	*Change in Output During Medication*	
							RL	*FS*
Arthritic	None	10	7.63	...	1.43	...	...	...
Arthritic	Oral cortisone	10	10.68	+40.0	2.61	+82.5	+3.05	+1.18
Schizophrenic	None	7	4.68	...	0.58	...	...	...
Schizophrenic	Cortisone I.M.	7	4.65	− 0.6	1.02	+75.9	− .03	+0.44
Arthritic	None	5	5.51	...	.71	...	...	...
Arthritic	Oral pregnenolone	5	5.22	− 5.3	.87	+22.5	− .29	+0.16
Schizophrenic	None	2	4.26	...	.59	...	...	...
Schizophrenic	Oral pregnenolone	2	5.01	+17.6	.56	− 5.1	+ .75	−0.03

that oral cortisone administration to arthritic subjects results in significant increases in urinary corticosteroid, whereas intramuscular injections of cortisone to schizophrenic men result only in an increased output of formaldehydogenic substance. Schizophrenic men tend generally to ex-

FIG. 5. The excretion of neutral nonketonic (NK) and ketonic (K) reducing lipids and formaldehydogenic lipids in arthritic subjects (A) and schizophrenic subjects (Ps) before (first rectangle of each pair) and during (second rectangle of each pair) cortisone acetate (CORT.) and pregnenolone acetate (PREG.) administration.

crete less urinary corticosteroid than normal subjects,[1-3] and this is again illustrated here. This tendency has been attributed to an endogenous hyposecretion, but since the schizophrenic men exhibit a diminished output after cortisone, it may be that they effectively destroy more cortisone *in vivo*. The fact that the formaldehydogenic substance increase during medication (76 per cent) was of the same order of magnitude as in the oral cortisone series (82.5 per cent) suggests rather a specific course of conver-

sion. It is clear also that total excretion figures would tend to mask any shift in excretion pattern, and further fractionation of these neutral lipids was undertaken.

Measurements of reducing lipids and formaldehydogenic substance have been made with the separated ketonic and nonketonic fractions of the neutral lipids during the premedication and medication periods for five arthritic men and five arthritic women receiving oral cortisone acetate, for six arthritic women and two arthritic men receiving oral pregnenolone acetate, for nine schizophrenic men receiving intramuscular cortisone acetate, and for the two schizophrenic men receiving 1 gm. per day of oral pregnenolone acetate. The mean data for these six groups are plotted in Fig. 5, in which the first of each pair of rectangles represents the output (in milligrams per gram of creatinine) during the premedication period and the second of each pair represents the output during the period of medication. The values for each sex are considered separately to determine if the fractionation might reveal differences concealed by the total values presented previously. The data of this figure demonstrate (1) increases in both ketonic and nonketonic corticosteroids in all subjects receiving cortisone, with the largest increases occurring in the arthritic subjects, and with minimal rises in the schizophrenic men in both reducing lipids and formaldehydogenic substance measurements; (2) no consistent change in the reducing lipids in the pregnenolone administration series, since the women show a measure of ouput decline during medication, the arthritic men show an output increase, and the schizophrenic men show no change; and (3) either no output change or a slight decline in formaldehydogenic substance measurements in all groups receiving pregnenolone. These data suggest that cortisone, especially when administered orally, induces the excretion of increased amounts of both ketonic and nonketonic corticosteroids; the fact that nonketonic steroids increase in amount suggests the transformation of cortisone, but the ketonic fraction increase might be due merely to the excretion of a moiety of the cortisone administered. The latter possibility may be tested by the further fractionation of the ketonic fraction.

Both fractions have, in fact, been chromatographed, and for purposes of demonstration in Figs. 6 and 7 we present reducing lipids and formaldehydogenic substance data on the two chief groups of eluates, numbers 2, 3, and 4 combined and numbers 6 and 7 combined. The former fractions contain less polar steroids and in the ketonic fractions tend to concentrate 11-desoxycorticosterone-like substances (in terms of polarity only); the latter fractions tend to concentrate compounds like corticosterone, cortisone, and 17-hydroxycorticosterone. The nonketonic steroids which would concentrate in the two sets of eluates are not specifically known, but the formaldehydogenic compounds would presumably be glycols, whereas the steroids with reducing activity might be unsaturated compounds having a degree of such activity, or α,β unsaturated ketones which fail to react with the Girard reagent used to effect the separation of ketones. In the early (nos. 2, 3, 4) nonketonic fractions, we might expect

to concentrate compounds such as Δ^1- or Δ^2-androstenol-17, whereas in the later fractions (nos. 6, 7), compounds like allopregnanetriol-3, 20, 21 might appear.

In Figs. 6 and 7 are charted the difference between the premedication and postmedication titers of the two sets of eluates in order to assess the type of substance excreted following the administration of a given steroid substance. Thus, in Fig. 6, we note that following oral cortisone the

Fig. 6. The change in output of the less polar (eluates nos. 2, 3, 4) nonketonic (open rectangles) and ketonic (cross-hatched rectangles) reducing lipids (RL) and formaldehydogenic lipids (FS) during the medication period as compared with the premedication period in arthritic subjects (♀ and ♂) and schizophrenic subjects receiving cortisone (CORT.) and pregnenolone (PREG.).

arthritic men and women both excreted notably increased amounts of nonketonic reducing lipids and more moderate increases of ketonic reducing lipids. The formaldehydogenic substance increases were also evident following oral cortisone. The schizophrenic patients who received intramuscular cortisone exhibited on the other hand only minor increases of reducing lipids and practically no increase of formaldehydogenic substance in these eluates. Following pregnenolone administration, a mean decline in all types of substances measured was observed in the arthritic women and the schizophrenic men, whereas moderate increases of both nonketonic and ketonic reducing lipids occurred in the arthritic men.

Considering the more polar fractions (Fig. 7), reducing lipid increases of nonketonic substances were obvious in both female and male subjects

receiving oral cortisone, but were less marked than in the early fractions (see Fig. 6); increases in ketonic formaldehydogenic substance are especially notable in these later fractions. The schizophrenic men in the cortisone series showed increases in all components, but again these increases were generally quantitatively less than those observed in the arthritic subjects. In the pregnenolone series, generally irregular small changes occurred with the exception of a clear increase in nonketonic reducing lipids in the schizophrenic men.

The suggestion of changes in the excretion pattern of urinary corticosteroids indicated by the foregoing data may be analyzed further by

FIG. 7. Data as in Fig. 6 but for the more polar chromatographic eluates (nos. 6 and 7).

examining the ratio of the components of the less polar to the more polar eluates in the various groups of subjects. The data so calculated are presented in Table II. As calculated, a value greater than 1 in these ratios indicates that the content of the less polar eluates (nos. 2, 3, 4) is greater than that of the more polar eluates (nos. 6, 7); if the ratio value is less than 1, the more polar material exceeds the less polar material in amount. For example, before medication the five arthritic women in the oral cortisone series tended on the average to excrete more nonketonic reducing lipids in the early eluates (ratio 2.2) and twice as much ketonic reducing lipids (ratio 2.0); the nonketonic formaldehydogenic substance of the early eluates exceeded that of the later eluates by over threefold (ratio 3.3), but the ketonic formaldehydogenic substance of the early eluates was less than that of the later eluates (ratio 0.83). If, during medication, there is a relative increase in early eluate materials, the ratio will rise, whereas if the later eluate material increases relatively, the ratio

will fall. Thus in both the arthritic women and men of the cortisone series there was a relative increase in early eluate nonketonic reducing lipids during medication (the ratios rose from 2.2 to 2.7 and from 2.4 to 3.4, respectively), whereas there was a relative decline in early eluate nonketonic reducing lipids in the cortisone-treated schizophrenic subjects (the ratio fell from 2.3 to 1.6). In fact, during medication, the cortisone-treated schizophrenic subjects exhibited uniformly lower ratios, suggesting the excretion of relatively more polar material throughout. Among arthritic patients receiving cortisone, three of the four formaldehydogenic substance ratios fell and the fourth (ketonic formaldehydogenic substance

TABLE II

THE RATIOS OF THE MEAN AMOUNTS OF REDUCING LIPIDS AND FORMALDEHYDOGENIC SUBSTANCE IN THE LATER CHROMATOGRAPHIC ELUATES (NOS. 6 AND 7) TO THOSE IN THE EARLIER ELUATES (NOS. 2, 3, 4) IN THE NONKETONIC (NK) AND KETONIC (K) FRACTIONS OF THE URINARY CORTICOSTEROIDS

			RL		*FS*	
			NK 2,3,4 / *NK 6,7*	*K 2,3,4* / *K 6,7*	*NK 2,3,4* / *NK 6,7*	*K 2,3,4* / *K 6,7*
Subjects	*No.*	*Medication*				
♀ Arthritic	5	None	2.2	2.0	3.3	0.83
♀ Arthritic	5	Oral cortisone	2.7	1.4	1.1	0.92
♂ Arthritic	5	None	2.4	1.8	1.7	1.0
♂ Arthritic	5	Oral cortisone	3.4	2.0	1.4	0.60
♂ Schizophrenic	9	None	2.3	1.9	1.1	0.84
♂ Schizophrenic	9	Cortisone I.M.	1.6	1.1	0.85	0.45
♀ Arthritic	6	None	2.9	2.8	1.2	0.91
♀ Arthritic	6	Oral pregnenolone	2.2	2.2	0.54	0.90
♂ Arthritic	2	None	1.1	1.1	0.54	0.70
♂ Arthritic	2	Oral pregnenolone	3.1	1.9	2.9	0.86
♂ Schizophrenic	2	None	3.8	2.2	2.3	1.1
♂ Schizophrenic	2	Oral Pregnenolone	1.3	1.5	0.73	0.64

in the arthritic women) rose only slightly, suggesting that more polar formaldehydogenic steroids tend to increase relatively; in contrast, three of the four reducing lipid ratios rose, indicating a relative increase in the excretion of the less polar reducing lipids in most instances.

In the pregnenolone series, the data for the two arthritic men stand out as exceptional, inasmuch as the premedication ratios were much lower than in all the other groups; the apparent return toward more "normal" ratios on pregnenolone administration, i.e. the excretion of a greater proportion of less polar substances, may have significance, but is difficult to assess until further data are at hand. In arthritic women and the schizophrenic men quite a similar pattern was observed, namely a reduction in the ratio in all types of analyses during the medication period. In these groups, therefore, pregnenolone medication was accompanied by a greater or lesser shift to the excretion of more polar substances both

ketonic and nonketonic and whether measured as reducing lipids or formaldehydogenic substance.

The indications of Table II that (1) the arthritic subjects receiving cortisone tended to shift toward the excretion of less polar reducing lipids and more polar formaldehydogenic substance, whereas the schizophrenic patients receiving cortisone shifted uniformly to more polar reducing lipids and formaldehydogenic substance, and (2) that pregnenolone in two groups caused a uniform trend to the excretion of more polar corticosteroids led us to examine our data on the most polar eluates (nos. 9 and 10). The data are presented in Table III, in which the reducing lipids and formaldehydogenic substance contents of eluate 9 plus 10 are expressed as a percentage of the total reducing lipids or formaldehydogenic substance in the nonketonic and ketonic fractions. Among the arthritic subjects there was a general tendency for the proportion of these most polar compounds to decline during cortisone therapy, with one exception (the nonketonic reducing lipids of the arthritic women). Among the schizophrenic men there was a similar but less marked tendency, but most notable was the uniformly higher proportion of these most polar materials in the schizophrenic men and the fact that during cortisone administration it remained even more elevated than in the arthritic series. In the arthritic women receiving pregnenolone these most polar eluates tended to increase relatively during medication, whereas the data for the arthritic and schizophrenic men showed more irregular changes during medication. Nonetheless, the two schizophrenic men exhibited high premedication levels, which were fairly well sustained during pregnenolone administration.

TABLE III

THE PERCENTAGE OF THE TOTAL RL AND FS IN THE LATEST CHROMATOGRAPHIC ELUATES (NOS. 9 AND 10) OF THE NONKETONIC (NK) AND THE KETONIC (K) FRACTIONS OF THE URINARY CORTICOSTEROIDS

			RL		*FS*	
Subjects	*No.*	*Medication*	*NK*	*K*	*NK*	*K*
♀ Arthritic	5	None	12.6	16.8	14.4	16.4
♀ Arthritic	5	Oral cortisone	12.8	12.6	8.9	7.0
♂ Arthritic	5	None	12.9	20.4	15.7	19.2
♂ Arthritic	5	Oral cortisone	11.6	10.9	15.1	11.0
♂ Schizophrenic	9	None	19.3	18.0	26.4	22.4
♂ Schizophrenic	9	Cortisone I.M.	17.3	19.0	23.1	18.9
♀ Arthritic	6	None	12.0	13.9	20.9	15.6
♀ Arthritic	6	Oral pregnenolone	15.3	14.0	26.2	18.0
♂ Arthritic	2	None	15.5	8.1	20.2	31.4
♂ Arthritic	2	Oral pregnenolone	7.5	12.8	17.9	12.6
♂ Schizophrenic	2	None	16.3	18.9	23.7	33.5
♂ Schizophrenic	2	Oral pregnenolone	15.9	22.1	24.1	28.4

What are the general implications of the corticosteroid excretion data? They suggest that arthritic subjects receiving oral cortisone tend to excrete increased amounts of nonketonic and ketonic reducing lipids less polar than cortisone, with accompanying reduction in the proportion of more polar reducing lipids. The predominance of a less highly oxygenated type of metabolite having reducing properties is indicated. This putative type of metabolite presumably does not have either an α-ketol or a glycol side-chain, since the formaldehydogenic substance data indicate a relative shift to material having cortisone-like polarity, and the accompanying reduction in the percentage of most polar formaldehydogenic substance (Table III) confirm the indication of this shift.

The data for the schizophrenic men have two notable features. First, they demonstrate a persistent high relative excretion of the most polar material and a fair maintenance of this level during cortisone administration. Second, the excretion shift following cortisone is to the cortisone-like materials in terms of polarity, when measurement is made of either reducing lipids or formaldehydogenic substance. The implication is that the corticosteroids ordinarily excreted by the schizophrenic men tend to be more polar substances than those excreted by arthritic men or women. The less polar substances are 11-desoxycorticosterone-like in polarity. They are relatively predominant in the arthritic patients. Is it conceivable that arthritis and this predominance are related? The schizophrenic subjects do not have arthritis and have a relative predominance of more polar substances. The tendency of the schizophrenic patients to excrete relatively larger amounts of material having cortisone-like polarity during medication may be due to the fact that they received cortisone by injection. In the case of the arthritic patients, the passage of the material through the liver may be responsible for the shift to a less polar reducing lipid metabolite, whereas liver-transformed metabolite is less evident in the schizophrenic subjects. Clearly the specific indentification of urinary corticosteroids following oral and intramuscular cortisone to arthritic and nonarthritic subjects would be most desirable, and we hope to have such data in the not-too-distant future.

The data on the effects of pregnenolone administration are less uniform, chiefly because of the peculiar premedication pattern exhibited by the two arthritic men. Nonetheless, although only minor quantitative changes in corticosteroid excretion occurred during pregnenolone administration, a shift toward the excretion of more polar corticosteroids is evident, and this shift may extend, in the case of the arthritic women at any rate, into the most polar fractions. If one might extrapolate the finding that pregnenolone is converted by the isolated adrenal to typical corticosteroid,[10] then a trend toward more polar corticosteroid would be expected in the human subject.

The Clinical Findings and the Biochemical Results

In the foregoing analysis no account has been taken of the clinical state of the subjects or of their response to administered steroid. Since

no therapeutic effect of cortisone or pregnenolone was observed in the schizophrenic patients they have served chiefly as controls for the arthritic patients. Their median age was 34.5 years, about one and one-half decades less than the arthritic subjects. In the case of the arthritic patients, however, two quite contrasting groups are concerned, particularly as regards clinical response to medication.

The 12 subjects receiving oral cortisone had a median age of 51.5 years and a median duration of the disease of 5.5 years; 11 of the 12 showed a degree of progression of the disease to put them in either stage 3 or 4 and from the point of view of disability 1 was classed as stage 2, 8 as stage 3, and 4 as stage 4. The degree of improvement observed after two weeks of oral cortisone was grade 2 in 8 subjects, grade 3 in 3, and grade 3 to 4 in 1. Thus, two-thirds of the patients showed significant therapeutic effect.[11] Six of these 12 subjects had previously had pregnenolone therapy, with responses graded as 3 in 4 subjects and grade 4 in 2 subjects. The 9 subjects receiving oral pregnenolone had a median age of 51 years and a median duration of the disease of 4.5 years; the degree of progression of the disease was to stage 2 in 3 patients, stage 3 in 3 patients; and stage 4 in 3 patients; the degree of disability was class 2 in 4 patients, class 3 in 3 patients, and class 4 in 2 patients. The response to medication was grade 2 to 3 in 1 patient, grade 3 in 5, grade 3 to 4 in 1, and grade 4 in 2. At best, 1 patient, or 11 per cent showed significant improvement.

We are thus presented with a group of patients receiving oral cortisone in which the majority showed significant clinical response, whereas the pregnenolone series showed only minor clinical response. We have stated that the biochemical data suggest that oral cortisone may be converted in the liver to a substance or substances which are 17-ketosteroid precursors having slightly salt-excreting and potassium-retaining effects, not markedly effective on uric acid or inorganic phosphate excretion, non-eosinopenic, lymphocytotic, and perhaps slightly hypercholesterolemic. Furthermore, this hypothetical substance would appear to affect glucose tolerance only mildly and to promote the degree of response to ACTH. In contrast, pregnenolone appears to be converted to a substance or substances which are 17-ketosteroid sparing, salt- and potassium-retaining, not markedly effective on uric acid or inorganic phosphate excretion, slightly eosinopenic and lymphocytotic, and perhaps hypocholesterolemic. Furthermore, this hypothetical substance does not affect glucose tolerance and promotes the magnitude of response to ACTH. If we take the effects which are common to oral cortisone and oral pregnenolone and rule them out as contributing to the clinical effect (since no notable response to pregnenolone was observed) then we emerge with marked improvement associated with 17-ketosteroid precursor having slight salt-excreting effect, non-eosinopenic, dubiously hypercholesterolemic and sugar-tolerance-decreasing. If we turn to our data on corticosteroid excretion, we find that both cortisone and pregnenolone tend to more polar urinary formaldehydogenic substance when eluates 2, 3, 4, and 6, and 7 are compared (Table II). They differ in that oral cortisone tends to increase the pro-

portion of less polar reducing lipids (Table II), and decreases the proportion of most polar reducing lipids and formaldehydogenic substance (Table III). Therefore our hypothetical substance or substances derived from oral cortisone is probably not cortisone-like but appears to act as precursor or precursors to materials appearing in urine which have reducing activity and rather low polarity. Dr. Zaffaroni may have the clue to these urinary substances. Our present interest is to conduct similar studies with patients showing significant clinical response to pregnenolone or other steroids to compare with this present study.

It may be alleged that the foregoing highly speculative analysis has little point. After all we have in cortisone (and presumably also in 17-hydroxycorticosterone) material that is markedly chemotherapeutic. Moreover in clinical effect, oral and parenteral cortisone appear to be equal. But our data show a wide divergence in the biochemical effect of each and that this wide divergence may be due to a transformation product of cortisone which acts efficiently to modify the course of arthritis, but lacks a number of the undesirable effects of cortisone (e.g. salt retention, pituitary inhibition). Why then don't we use oral cortisone therapy as the mode of treatment? This question cannot be answered with the data at our disposal, but the effects of protracted therapy with oral cortisone require further investigation. Perhaps the liver-conversion mechanism postulated may be eventually overwhelmed or allow escape; perhaps more direct cortisone effects will eventually appear. Most appealing to us as investigators is the testing of the hypothesis we have deduced from the data as they stand.

Acknowledgments

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Discussion

Owing to unforeseen circumstances, Dr. Pincus was unable to attend the symposium. Therefore no discussion on his paper took place.

9

Adrenal Function and Steroid Excretion in Disease

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We have studied steroid excretion patterns in man as a means of defining the details of hormone production by the adrenals and gonads.[1] The evidence which correlates the metabolism of a normal or abnormal adrenal hormone production in neoplastic disease, essential hypertension, and arthritis will be discussed and compared with the findings in healthy subjects. The capacity of the adrenal to respond to stimulation as well as the type and character of the response will be dealt with as a part of this program.

If we examine the steroid excretion patterns of normal men and women (Fig. 1) we find there are four compounds that make up the major part of the ketosteroids. Two of these are unquestionably derived from adrenocortical hormones with a C11-oxygen function; androsterone and etiocholanolone are derived from C11-desoxy steroids from both gonads and adrenals. In addition to these major metabolites, small amounts of many other steroids are excreted,[2, 3] the amount of each compound varying somewhat in different individuals. In normal subjects there are no qualitative deviations from the normal pattern; there are quantitative differences between individuals just as there are quantitative differences in any metabolic process. Nevertheless each individual has a pattern of steroid excretion which during comparisons over periods longer than one year remains very constant.

The steroid pattern is quite abnormal in neoplastic disease.[4] This is illustrated in Fig. 2, where a comparison of the excretion levels of normal subjects of similar age has been made with the neoplastic patients. There are quantitative differences which lead to the disappearance of some normal constituents, but of much greater importance is the fact that in a significant number of patients with neoplasia there are abnormal compounds exemplified by 11-hydroxyetiocholanolone. We have concluded from these results that there is an abnormal adrenal function or an altered metabolism of adrenal hormones when cancer is present. The evidence has been discussed elsewhere in detail,[4] and is derived from the consistent occurrence of 11-hydroxyetiocholanolone in the urine of patients with

the adrenal dysfunction called Cushing's syndrome. That this change in steroid excretion was present *before* cancer of the breast was diagnosed is shown in Fig. 3. The presence of the abnormal pattern persisted as shown for three years after successful operation, and the atypical compound and pattern were still found after five years, although this is not charted. The patient is well and has had no recurrence of the disease. In Fig. 4 are

FIG. 1. Steroid excretion in normal male and normal female subjects.

shown the excretion patterns of the same steroids in women with essential hypertension. We received the urines through the co-operation of Drs. W. Goldring and Homer W. Smith of New York University College of Medicine. This study was made to determine whether abnormal production or metabolism of steroid hormones existed in essential hypertension. In a significant number of these patients again, the abnormal metabolite, 11-hydroxyetiocholanolone, was present, and in addition an-

other steroid of undetermined structure was found in the urines of all of these patients. The conclusion can be drawn that in this syndrome a disturbance of hormone production and/or metabolism is present.

The status of adrenal function is of current special interest in arthritis, a condition which responds so strikingly to the administration of cortisone and Compound F as well as to the stimulation of the adrenals by ACTH.[5] Adrenal function in arthritis has been much discussed but little investigated. Through Drs. Sprague and Mason of the Mayo Founda-

FIG. 2. Comparisons of steroid excretion patterns in patients with neoplastic disease and in normal subjects of the same age.

tion we have obtained the urine of a patient with arthritis and spondylitis. The excretion pattern of the urinary steroids in this patient is shown in Fig. 5 in comparison with that of a normal male. The amount of urinary steroids in the arthritic patient is actually at the lowest level found in normal subjects. Pregnanolone is excreted by the normal male but is absent in the arthritic patient. The most significant finding is the excretion of 17-hydroxypregnanolone in the patient with arthritis. This compound, from its characteristic structure, is a metabolite of an adrenal cortical hormone. Since this compound has been found quite regularly in adrenal hyperplasia and adrenal tumors and never in the urine of 28

normal subjects, the conclusion can be drawn that this arthritic patient had an adrenal dysfunction. It remains to be determined whether adrenal dysfunction can be confirmed in other cases of arthritis.

FIG. 3. Steroid excretion in cancer of the breast, showing abnormal pattern prior to diagnosis persisting after successful operation.

To study further the adrenal function of normal and diseased persons, we have administered ACTH to a group of neoplastic patients as well as to normal subjects.[5-7] Our intent was to stimulate the adrenal and

to ascertain whether adrenal function can be restored toward normal. It was also important to determine whether the adrenal in diseased persons

FIG. 4. Steroid excretion patterns in normal women and in women with essential hypertension.

responded in the same way as in normal individuals. In six normal males and nine subjects with neoplastic disease the ketosteroid and formalde-

hydogenic steroid levels were measured for a control period. Following this, all the subjects each received 100 mg. of ACTH in four divided doses for 12 days. In five normal subjects the increase in ketosteroid and formaldehydogenic steroids was remarkably similar (Table I). In one

FIG. 5. Steroid excretion in a patient with rheumatoid arthritis.

subject no increase of ketosteroid excretion was observed, whereas his increase of formaldehydogenic steroids was within the range of the other normal men. This individual, in metabolic studies made by Dr. Shock and his co-workers,[8] showed no striking change in his nitrogen and phosphorus excretion but did show a change in electrolyte balance similar to the other normal subjects. Analogous differences in response to ACTH stimulation have been reported by us in several patients with neoplastic disease.[6, 7] In metabolic studies by Drs. Pearson and Eliel,[9] patients with only a slight increase of ketosteroid excretion showed only a slight change

TABLE I

KETOSTEROID AND CORTICOSTEROID EXCRETION AFTER ACTH INJECTION IN NORMAL SUBJECTS

Ketosteroids (mg./24 hr.)		*Formaldehydogenic Steroids (mg./24 hr.)*	
Control	*12 Days ACTH*	*Control*	*12 Days ACTH*
22.7	58.1	0.3	8.1
20.2	60.7	0.4	12.4
23.1	29.8	0.2	7.1
15.0	54.0	0.3	14.1
17.4	55.9	0.2	11.9
21.0	39.7	0.1	5.5
AVERAGE			
19.9	49.7	0.3	9.9
RATIO			
1.0	2.5	1.0	34.7

in nitrogen and phosphorus excretion. If one compares the steroid response after ACTH of the six normal subjects (Table I) with that of the patients with neoplastic disease (Table II), it is clear that ketosteroid response in both groups is of the same order of magnitude. In contrast to this there is a striking difference in the excretion of formaldehydogenic steroids (Table III). These levels in neoplastic disease during the control period are higher than those in the normal subjects (indicating a state of

TABLE II

KETOSTEROID AND CORTICOSTEROID EXCRETION AFTER ACTH INJECTIONS IN PATIENTS WITH NEOPLASTIC DISEASE

Ketosteroids (mg./24 hr.)		*Formaldehydogenic Steroids (mg./24 hr.)*	
Control	*12 Days ACTH*	*Control*	*12 Days ACTH*
14.7	63.6	4.2	11.9
16.6	42.7	1.7	37.8
17.0	41.7	5.4	24.0
24.7	32.6	1.0	18.3
16.5	38.6	2.4	25.5
10.7	48.6	..	...
16.0	60.8	10.5	40.4
15.1	55.0	0.2	11.9
17.1	74.9	5.0	43.0
AVERAGE			
16.5	50.9	3.8	26.6
RATIO			
1.0	3.1	1.0	7.0

alarm), and during stimulation of the adrenals with ACTH the values reach levels twice those of the normal subjects. This definitely indicates a different functional status of the adrenal in the patients with neoplasia. The results show in addition that although the adrenals are already functioning at an increased level in the diseased patients, a pronounced response to adrenal stimulation is still readily achieved and indeed the response is greater than that of a normal gland.

A study of individual steroids excreted before and during stimulation of adrenal function by ACTH should give more information on hormone production and metabolism. In Fig. 6 the patterns of the most abundant urinary steroids before, during, and after ACTH administration in three

TABLE III

COMPARISONS OF KETOSTEROID AND CORTICOSTEROID EXCRETION AFTER ACTH INJECTIONS IN NORMAL SUBJECTS AND IN SUBJECTS WITH NEOPLASTIC DISEASE

	Ketosteroids (mg./24 hr.)		*Formaldehydogenic Steroids (mg./24 hr.)*	
	Control	*ACTH Stim.*	*Control*	*ACTH Stim.*
		AVERAGE		
Normal (6)	19.9	49.7	0.3	9.9
Neoplastic Disease (9)	16.5	50.9	3.8	26.6
		RATIO		
Normal (6)	1.0	2.5	1.0	34.7
Neoplastic Disease (9)	1.0	3.1	1.0	7.0

patients with neoplastic disease is shown with the pattern of normal subjects of the same age group without treatment. There was an increased excretion of both 11-oxygenated and 11-desoxy steroids during adrenal stimulation.[7, 10] The several patients responded with different amounts of some steroids and in addition new compounds appeared; and these, too, were not the same in all subjects. These results indicate qualitative and quantitative differences in responsiveness of individual patients to adrenal stimulation.

The excretion patterns of six ketosteroids after ACTH and cortisone administration are shown in two patients with lymphatic leukemia (Fig. 7). The patient who received cortisone excreted only trace amounts of the six compounds during his control period. After administration of cortisone (100 mg. for six days and 200 mg. for 12 days) a marked increase of the two 11-oxygenated urinary metabolites occurred, and *no* increase of the 11-desoxy metabolites was observed. This result is in sharp contrast to

FIG. 6. Effect of ACTH administration on urinary steroid patterns in normal subjects and in patients with neoplastic disease.

the great increase in excretion of *both the 11-oxygenated as well as the 11-desoxy urinary steroids after ACTH*. These findings are of great significance in relation to the hormone production of the human adrenal. The following conclusions can be drawn:

1. Since after the injection of large amounts of cortisone no increase

FIG. 7. Steroid excretion after ACTH or cortisone administration in lymphatic leukemia.

of 11-desoxy steroids is found in urine, *cortisone and other C11-oxygenated* adrenal hormones, such as *Compound F, do not contribute to the excretion of 11-desoxy steroids.*

2. Since after the injection of cortisone an increase of 11-oxygenated C19 steroids occurs in urine, *11-oxygenated adrenocortical hormones are*

FIG. 8. Effect of prolonged testosterone injection on steroid excretion pattern.

the precursors of these urinary metabolites regularly found in the urine of normal subjects.

3. Since after stimulation of the adrenals by ACTH 11-oxygenated as well as 11-desoxy steroids are excreted in increased amount in the urine, *the human adrenal produces 11-desoxy hormones as well as 11-oxygenated hormones.*

Time does not permit a full discussion of the mechanism of the

alteration in hormone production and metabolism which occurs after stimulation of the adrenals with ACTH and after the administration of cortisone. Such compensatory alteration has been discussed recently in connection with gonadectomy and ovariectomy and the administration of such steroid hormones as testosterone, progesterone, and adrenal extracts.[1] We have shown that the hormonal balance can definitely be influenced by these procedures. I should like to illustrate this by two experiments.

FIG. 9. Steroid pattern as influenced by adrenal cortical extract injections.

The first is the effect of prolonged testosterone administration (90 mg. testosterone daily for 45 days) on the excretion of androsterone and etiocholanolone in a pre-injection control period and in the post-injection period shown in Fig. 8. The excretion of androsterone and etiocholanolone was *significantly* decreased in the post-injection period. A similar change in steroid excretion is seen in Fig. 9, after the administration of 5 ml. aqueous adrenal extract (Upjohn) for 50 days; the steroid excretion fell to a very low level, and returned to nearly pretreatment levels after cessation of the injections. These effects can best be explained as a change in the regulatory mechanism of the pituitary secretion of trophic hormones influencing the adrenals and the gonads. The clinical response to the continuous administration of steroids in neoplastic disease, rheumatoid arthritis, and other conditions may have its explanation in these indirect changes of hormone production and hormone balance. An ex-

cellent illustration of a compensatory alteration is the physiologic adjustment to hormone production during pregnancy. As seen in Fig. 10 and described in detail elsewhere,[1, 11] concomitantly with the great production of new steroids evidenced by pregnanolone and its isomer, allopregnanolone, there occurs in the later part of pregnancy a decreased excretion of androsterone and etiocholanolone. Furthermore, an 11-oxygenated ster-

FIG. 10. Steroid excretion in pregnancy.

oid, 11-hydroxyandrosterone, disappears from the urine during the last six months of pregnancy. A normal excretion pattern is again found post-partum.

The hormone production and excretion during pregnancy likewise illustrate an important and all too frequently mistaken mental attitude toward steroid therapy. *The administration of large amounts of steroids is not necessarily unphysiologic.* It has been shown that the increase in hormone production during pregnancy is at least 50 to 100 times that of the nongravid state. This large increase is necessary for a purely physiologic process and is achieved without difficulty and certainly without harm to the woman. In order to obtain pregnanolone excretion comparable to that of pregnancy by progesterone administration to a non-pregnant subject, it would be necessary to inject more than 1 gm. per day. The same consideration applies to the excretion of estrogens, gonadotrophins, and glycogenic[12] steroids.

Not only the amount of steroid but the individual effect of several hormones and the combined performance of diverse synergistic and antagonistic operations, as well as the sequential quality of these activities, are significant features of the endocrine function in pregnancy. Together with this altered production of steroids there is more than likely a difference in tissue response to the hormones and possibly a change in metabolic path. These phenomena are but dimly perceived in the light of our present knowledge but there is no doubt in my mind that the beneficial effect of pregnancy in arthritis is due to the high level of steroid hormone production and an adjustment of the organism to a new balance in metabolic processes, regulated by the complicated interplay of these forces. The overwhelming importance of the hormones is demonstrated by the prompt recurrence of arthritis post-partum when the support of the high hormonal level has been withdrawn. It is wholly reasonable to speculate and imperative to investigate what factors in the steroid hormone production and metabolism produce these physiologic changes in the arthritic patient. We should thus find a guide to a rational therapy of this syndrome, an insight into the mechanism of the disease, and a most significant advance toward understanding the action of hormones.

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Discussion

R. I. Dorfman: I would like to relate some of our experiences with the metabolism of progesterone and related compounds in rheumatoid arthritic patients, which appear to be related to Dr. Dobriner's observation on the absence of pregnanolone in the urine of the rheumatoid arthritic patient he studied. In our work we administered pregnanedione-3,20 which should be converted to both pregnanolone and pregnanediol. We were able to account for 7.8 per cent of the administered steroid as pregnanediol but we were unable to isolate pregnanolone. It would be of interest to extend these studies to normal individuals.

With respect to the metabolism of pregnanolone, we have been able to show that this steroid in rheumatoid arthritic men is converted to Δ^5-pregnenediol-3β,20 as well as to pregnanediol. Here again no pregnanolone could be demonstrated. Dr. Dobriner has presented very interesting data on the changes in steroid excretion in his rheumatoid arthritic patient. We shall look forward to additional data on this subject.

R. A. Davison: I would like to add two more documented cases of spondylitis to Dr. Dobriner's one in which we have found an abnormal pattern of ketosteroid excretion. While we have not identified fully the compounds which have been excreted, the pattern is quite similar, although of lesser magnitude than the pattern we have obtained in a case of proved adrenocortical hyperplasia.

A. White: I wonder if it is valid, until such time perhaps when we can perfuse isolated adrenals taken from diseased individuals, to conclude unequivocally that the data presented by Dr. Dobriner are evidence of an abnormal production of adrenal cortical steroids? Is there not the possibility that these abnormal urinary steroid patterns are an indication of an altered manner or manners in which peripheral tissues metabolize the normal adrenal steroids produced by the adrenal cortex? In other words, is the evidence clear that these abnormal steroids are being produced by the adrenal cortex rather than that they may arise in diseased states from aberrant reactions occurring in tissues other than the adrenals?

K. Dobriner: Let us take for example the finding of a 17α-hydroxypregnanolone in the urine of an arthritic subject as a means of answering Dr. White's question. We know that this compound is a metabolite, as distinct from a primary secretion product of the gland. We have never found this steroid metabolite in the urine of a normal person. We have found it, however, in such conditions as adrenal hyperplasia of the virilizing type, adrenal tumors, and after the stimulation of the adrenals with ACTH. We know and recognize that the adrenal function in these conditions is abnormal. The conclusion is justified, therefore, that the primary disorder is in the adrenal and not in the metabolism of the adrenal hormones by the peripheral tissues.

I. T. Nathanson: Dr. Lewis Engel and I, using a somewhat different approach from Dr. Dobriner and his colleagues, are also attempting to detect differences in steroid metabolism among patients with various disease states. These studies complement each other. Our plan is to administer various dosages and classes of steroid and pituitary compounds to ascertain if there is a difference in the relative excretion rates and patterns of the urinary steroids. For this purpose we use a battery of techniques which include estrogens, 17-ketosteroids, nonketonic alcohols, and adrenocortical metabolites. To date in a study of about 15 patients it is clear that with dose levels of ACTH varying between 50 and 100 mg. per day over relatively short periods, the excretion rates and patterns of these various steroid metabolites vary considerably from patient to patient. These data coincide very well with the excellent studies of Dr. Dobriner and his group. This suggests that there is either a significant difference in the receptivity of the adrenal glands of the different patients to a given dose of ACTH or, as Dr. White suggested, differences in the metabolism of the secreted compounds at the peripheral level. In addition to determinations

of the total steroid excretion, we are attempting to identify the individual components of each steroid complex. At present our efforts are confined primarily to the separation of the estrogens, for which we have employed successfully the techniques of countercurrent distribution. Our studies include also the steroid excretion patterns after the administration of steroid hormones. An example of this approach is given in our paper in this symposium.

I should like to ask Dr. Dobriner how satisfied he is with the techniques of hydrolysis and extraction of the urine for the various steroid complexes. We are particularly unhappy with the methods employed to estimate the formaldehydogenic and reducing steroids, although the same comment applies to the other steroids.

F. Gomez Mont: We have been studying, during the past three years, the endocrine changes in malnutrition. We have found a very low excretion of 17-ketosteroids in all these patients. In many of them we gave testosterone propionate in doses of 25 to 100 mg. daily for long periods of time without being able to find a corresponding increase in the 17-ketosteroid excretion. We think that in malnutrition there is an abnormal metabolism of testosterone. This is probably one of the factors contributing to an explanation of the low excretion of 17-ketosteroids.

I would like to know if Dr. Dobriner's studies were done in malnourished people.

K. Dobriner: I agree with Dr. Gomez Mont that there is a lowered ketosteroid excretion in malnutrition, and I would further extend this statement and emphasize that there is a lowered ketosteroid excretion in debilitated persons. I believe that there is a lowered production of steroid hormones by these individuals. This lowered production may be further complicated by alterations in metabolism of the hormones, so that no clear-cut distinction can be drawn between production and metabolism. I have an experiment which clearly demonstrates that in a normal man, acute biotin deficiency changed his urinary steroid excretion pattern. The subject was a young physician who received massive amounts of avidin with a low biotin intake. His steroid excretion prior to and during the experimental period are shown in *Fig. 1*. There was a definite decrease in the excretion of C11-oxygenated steroids during the experimental period.

I would like to congratulate Dr. Zaffaroni for this beautiful piece of work he has reported, particularly with respect to techniques and concept. I think this is the second time, considering the work of Bloch and Rittenberg, that cholesterol has been shown to be the starting product in the biosynthesis of a C21 steroid. I may further report that Dr. Gallagher, Dr. Fukushima, and I can contribute additional information to this problem in the human. We have been fortunate to have co-operated with Dr. Peacock and Dr. Hellman in the study of patients fed with carbon-14-labeled acetate. We have compared the radioactivity of the serum choles-

Fig. 1

terol with one of the urinary steroids, namely dehydroisoandrosterone. We have found that the urinary dehydroisoandrosterone was directly derived from cholesterol since the activity was the same and the decline of specific activity was the same as that of the serum cholesterol. Dehydroisoandrosterone, for the nonchemically minded members of the conference, is nothing else than cholesterol which has undergone castration of the side-chain.

A. Segaloff: In our laboratory and at Sloan-Kettering under Dr. Reifenstein and Dr. Escher it has been found that in many patients with breast cancer there is a derangement in pituitary gonadotrophic hormone excretion. It would appear attractive in view of Dr. Selye's comments this morning that possibly the apparent derangements of adrenal function could be related to a derangement of pituitary function.

K. Dobriner: We have talked about adrenals and adrenal steroids, and we forget about the regulating mechanism of the hypophysis. The reason we don't talk about it is that we have no decent methods to measure the

trophic hormones. I have not been involved in the studies of gonadotrophic hormones in those tumor patients and I would like to have Dr. Reifenstein answer Dr. Segaloff's question.

G. Sayers: If I may attempt to answer the question, in cancer and in other pathological situations one would expect a stimulation of the pituitary-adrenocortical axis. One might see dysfunction with regard to gonadotrophic function and dysfunction with regard to adrenocorticotrophic function. In a cancer patient, for instance, there is a dysfunction of the adenohypophysis.

E. C. Reifenstein, Jr.: The studies I initiated at the Sloan-Kettering Institute on the gonadotrophin excretion by postmenopausal women with cancer of the breast are being continued by Dr. George C. Escher. I made a very preliminary analysis of the data some months ago, which really was not based on a sufficiently large number of patients to warrant more than some vague impressions. It appeared at that time that a large number of the postmenopausal women with cancer of the breast had a rather low excretion of gonadotrophin in the urine. Consequently we raised the question as to whether or not this decrease was a specific or a nonspecific effect. We examined the gonadotrophin excretion of a series of postmenopausal women who were bed-patients with non-malignant diseases, and found that they also had a low excretion of gonadotrophin. The analysis suggested that the gonadotrophin excretion was elevated in only about 50 per cent of postmenopausal women with non-malignant disease who were bed-patients, and in only 25 or 30 per cent of ambulatory postmenopausal women with cancer of the breast. These results were quite in contrast to the findings in a series of about 50 postmenopausal women who were ambulatory and who visited the clinic for relatively minor complaints; of these, about 80 per cent had an elevated gonadotrophin excretion. It looked as though there might be a lowering of gonadotrophin excretion due to nonspecific factors, and the question was raised as to whether or not there was some specific effect on gonadotrophin excretion in neoplastic disease. This question cannot be answered from our data at present; we feel it is an important problem that should be studied further.

We have not measured the production of adrenocorticotrophic hormone in such patients, but we have made this interesting observation. Male patients with gastric cancer show some unresponsiveness to adrenocorticotrophic hormone by certain indices, when compared with a group of males of the same age with non-malignant diseases. The gastric cancer cases did not respond with a rise in the urinary uric acid-creatinine ratio or a rise in the urinary phosphorus-creatinine ratio with a given dose of ACTH, which produced a rise in these urinary ratios in the patients with non-malignant diseases. However, the gastric cancer cases did show the same rise in the urinary potassium-creatinine ratio and the same fall in eosinophils after the same dose of ACTH as did the non-malignant cases.

These differences are apparent only when patients of the same sex and age are compared. There appeared to be some dissociation in response to ACTH in the patients with gastric cancer, which suggested that the adrenal cortex was somewhat unresponsive. We have no knowledge as to whether or not the pituitary is involved in this alteration in adrenal responsiveness.

C. Sayers: I can see that we are still involved in the problem of how much to blame the endocrine gland and how much to blame "utilization" and/or degradation of the endocrine secretory products in the peripheral tissues themselves. It is a difficult problem to solve. Furthermore, in using metabolic changes as indices of adrenocortical function, one must be aware of the fact that peripheral target cells may respond in a variety of ways depending upon the circumstances. A less than normal metabolic response may be due to changes in the target organ, not in the secretion of the adrenal cortex.

J. W. Conn: If one assumes, Dr. Dobriner, that the variation in the steroid excretion pattern of cancer patients is the result of changes in adrenal cortical activity, then it is of interest that in the cancer patients the formaldehydogenic steroid excretion seems to be somewhat higher than in the normal subjects, while the ketosteroids are at the normal level. That is reminiscent of the situation obtained in a normal individual on the fourth or fifth postoperative day.

There seems to be increasing evidence that the response to administration of ACTH is not wholly comparable to the alarm reaction *per se*. In other words, the alarm reaction produces some of the responses that occur upon the administration of ACTH, but the whole integrated alarm is probably more than ACTH alone. Now, I would like to ask Dr. Dobriner if he has had the opportunity to do these careful steroid excretion patterns on a normal individual and then to repeat them following major surgery or after severe injury, to see whether the excretion pattern changes in a previously normal individual who has been injured. I think that the answer to that question could be important for this reason. Is the excretion abnormality the result of the disease, or the cause of the disease? If the excretion pattern changed in a normal person under circumstances of stress, one then might assume that the changes that are being observed in disease are the result of the disease rather than its cause.

K. Dobriner: Until some time ago it was not possible to establish a urinary steroid pattern without collecting urine for 30 or more days; in some of the cancer patients we had to do collections of 100 to 200 days. By the application of infrared spectroscopy, we have been able now to cut the collection periods down in a normal person to one day, if we like. We have studies in progress to answer exactly these questions. One of your other questions; is the urinary steroid pattern the cause or the result of the disease? is a difficult question. However, we have had some lucky

experiences with two patients whom we have studied. I had a technician whose urine I collected four years before cancer of the breast was diagnosed, and four years before this diagnosis was made there was already an abnormality of her steroid pattern. I have been able to follow her case for the last five years after operation. She is a person who has not lost a gram of weight; certainly no obvious stress is involved, and she still has her abnormal pattern. In the second instance, we had the opportunity to collect the urine of a woman in the late thirties in the Rockefeller Institute for aplastic anemia. I got the urinary steroid pattern about four years after the urine was collected and this was an abnormal pattern. At that time we said that if there is in aplastic anemia a steroid pattern similar to that seen in the cancer patient, then the value of these patterns may be questionable. In tracing this patient, we found she had died of cancer of the lung. The urine had been collected four years before death and certainly no diagnosis of lung tumor was made at that time and I suppose no one can say whether at that time she already had a tumor. There is certainly a certain suggestion, at least in my mind, of some positive causal relationship between pituitary-adrenal function and malignancy.

A. White: There are animal experiments which suggest a correlation between at least alterations in adrenal size and the induction of malignancy. I refer to the experiments of Gardner, Dougherty, and Williams in which it was demonstrated that the development of a lymphoid tumor in mice as a consequence of the implantation of estrogen pellets appeared to be correlated with adrenal size as follows: There seemed to be a critical period of approximately nine weeks during which it was necessary for the estrogen pellet to remain in place in the animal. The incidence of malignancy was about the same whether the estrogen pellet was removed at any time after nine weeks or was permitted to remain in the animal. In other words, there was a critical nine-week induction period. During that nine-week induction period there was a continuing increase in adrenal size. At nine weeks there was a reversal in the growth or enlargement of the adrenal with a subsequent adrenal atrophy during the month which followed, so that at least in time and in adrenal size there was this apparent relationship to malignancy development.

K. Dobriner: I'd like to add that in the experiments of George Wooley in genetically controlled strains of mice there appears to be a positive correlation between adrenal size and malignancy.

10

Introduction and Statement of Problems Relative to Hormonal Therapy in Arthritis

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I understand that today has been designated for consideration of the rheumatic diseases, particularly rheumatoid arthritis. I shall try during these few opening remarks to avoid, insofar as possible, facts and shall, rather, discuss something of our attitudes and goals.

Steroid chemistry has become in recent years the Cinderella child and the magic "Open Sesame" to much knowledge and even higher future hopes in many fields of medicine. It was less than 20 years ago that the structure of the steroid nucleus was finally determined. Perhaps my only claim to knowledge in this field, though very slight indeed, is due to the fact that while in college I once wrote a thesis entitled "Cholesterol, the Source of Life." Incidentally, I received a low grade for my effort. The professor noted on my paper, "Not enough facts, mostly imagination."

During recent years it has become common knowledge that sex hormones, bile acids, vitamin D, digitoxin, adrenal steroids, and even toad poisons are closely related chemically. It is known that slight changes in this steroid nucleus, such as adding different side-chains at C17, changing the degree and position of unsaturation, and providing variations of stereoisomeric forms completely alter the biologic action. The total number of theoretically possible variations would produce a fabulously large number of different compounds.

Interest in the steroids as possible antirheumatic agents has existed for many years, as evidenced by the use of vitamin D, estrogens, androgens, and bile acids as therapeutic agents. This interest received a tremendous impetus with the demonstration by Hench and his co-workers that cortisone, an 11-oxysteroid, would produce dramatic remissions in rheumatoid arthritis. Only a few of the other related steroids have been available for study. Many conflicting reports have appeared in the literature during recent years regarding their effects in rheumatoid arthritis. Extremely divergent points of view have been expressed. Reports have come from men whose honesty and ability cannot be questioned. Yet we, and I am sure that some of you, have often been unable to confirm their results.

It should be clear at this point that one must ask oneself, what is wrong with this picture? Now there are two possible ways to answer this question. One is to say, "I tried it and did not get such results. Therefore it is worthless." This is indeed a tempting, and certainly the easiest, way out of the dilemma. I am sure we have all been guilty of this at some time.

The other way to resolve the difference is to seek out every variation between the two studies performed. There may be differences in the type of patient, dosage, length of administration, degree of remission, method of evaluating improvement, etc. I think it is axiomatic to say that variations must exist or the results would be identical. In our evaluation of therapy and in our search for the remission factor, we should remember that just as patients vary widely in the ease with which remission is induced, just so test substances may vary in strength or concentration of remission factor. It is certainly conceivable that a substance may be only 10, 20, or 50 per cent as powerful as cortisone, but at the same time be capable of producing some degree of remission. Likewise, of course, a substance may be found many times as potent as cortisone. However, nothing in the rules specifies that to induce remission, a substance must produce dramatic changes in seven days.

If the final answer to the treatment of rheumatoid arthritis were at this moment known, there would be no reason for this session of the conference. I am sure that you will all agree that even with the great advances made in treatment, using cortisone and ACTH together with all other methods available to us, there are still a large number of patients whose disease cannot be controlled safely by known methods. Actually, we have as yet no satisfactory knowledge of the mechanism of the disease and know little or nothing regarding the site and manner of action of our therapeutic drugs. The nature and mechanism of the remission factor, or Factor X, remains as great a riddle as ever. The identity of Factor X will bear some speculation. That remission in rheumatoid arthritis may occur with cortisone ACTH, gold, pregnancy, jaundice, occasionally with transfusions, and sometimes spontaneously, is well known. One must, therefore, assume that if the remission factor is a specific mechanism, it should be constantly associated with remission from whatever initiating cause.

The alternative is to propose that there are several different remission factors. If we think of this problem at the cellular level, it appears more probable that the chemical or enzymatic change producing remission is the same in all cases. With this hypothesis, it would appear logical to seek for Factor X as a common denominator in remissions from all causes. Much work has been done on the metabolic changes occurring during the administration of ACTH and cortisone. So far, not a single metabolic change measured has been found constantly associated with, and essential to, remission. There is no evidence to show that gold or jaundice remissions exhibit any of the hormonal effects of cortisone or ACTH. It seems fair, then, to conclude that while ACTH and cortisone in some unknown manner may initiate the remission factor, the remission factor itself is

separable from, and not dependent upon, the known hormonal effects of ACTH and cortisone. Cortisone is an 11-oxysteroid capable of initiating remission. The long-term maintenance of remission may be difficult, and in many patients impossible, because of its hormonal effects. Until we know how cortisone initiates a remission it is unlikely that we shall be able to tell from the chemical formula whether a substance will induce the remission factor or not. We are, therefore, continuing the search for a steroid or other substance capable of producing remission in any degree without exhibiting unfavorable hormonal effects. This thinking, then, has been the basis of our interest and willingness to study other steroids and their effects in rheumatic diseases. I am sure that this conference will add much to our knowledge of this most important subject and, possibly, to our understanding of each other.

11

The Prolonged Use of Testosterone and Pregnenolone in the Management of Chronic Rheumatoid Arthritis

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Pronounced alterations in the arthritic state often occur concomitantly with general metabolic changes which may be associated with gonadal activity. For many years physicians have recognized the ameliorative effect of pregnancy, the flareups associated with the menstrual period, the onset or relapse of rheumatoid arthritis following pregnancy, the onset or exacerbation of the disease with menopause or oöphorectomy, and the "burning out" of the arthritis following menarche or puberty. In addition, the onset of menstruation is usually delayed in girls with Still's disease until the age of 18 or 19. Furthermore, there is a sex distribution in rheumatoid variants; rheumatoid spondylitis occurs primarily in males, whereas "typical" rheumatoid arthritis is predominant in females.

In the past the use of estrogens and androgens as a treatment for rheumatoid arthritis has been disappointing. On the other hand, degenerative joint disease, particularly that following the menopause or associated with aging, responds well to certain "sex" steroids.[1-3] Estrogens have been used for postmenopausal arthritic states since 1937.[4-7] After several years, it was suspected that the beneficial effect exerted was not through its hormonal "sex" action but rather through some more basic metabolic function. As a result, androgens, alone and in combination with estrogens, were administered for degenerative joint disease regardless of sex. Occasionally a rheumatoid patient with associated degenerative changes would respond rather gratifyingly when these steroids were administered in what were then thought to be physiological doses.

The first steroid administered in relatively large doses to patients with rheumatoid arthritis was activated ergosterol.[8] Some patients responded favorably. Smaller doses resulted in no such beneficial effect. The hyper-

* We wish to express our appreciation to Miss Opal Betty Rittersbacher for her valuable assistance in the preparation of this paper.

calcemia and hypercalciuria and the complications resulting from these two disturbances limited the use of this substance.

We reported the results of the administration of relatively large doses of testosterone and pregnenolone in October 1949.[9] In further studies, an additional 100 patients with rheumatoid arthritis were treated with testosterone and pregnenolone. The results obtained from the second group of patients, with the follow-up studies on the original group, were reported at the 1950 meetings of the American Rheumatism Association in San Francisco.[10] On that date the original group of patients had been under treatment for an average of approximately one year. The present report presents a continuation of the previous studies and deals with the more prolonged effects of testosterone and pregnenolone in the same 190 patients for as long as 18 months.

Other investigators have observed beneficial results following the administration of testosterone in rheumatoid arthritis. Howard, Venning, Fisk and Fay[11, 12] at the McGill University Clinic of the Royal Victoria Hospital reported subjective improvement in 8 of 10 patients, but definite objective improvement in only 5. Improvement was maintained for 2 to 6 weeks after testosterone treatment was stopped. Therapy was reinstituted in 2 patients with remission in both.

Col. Ralph M. Patterson[13] of the Army and Navy General Hospital at Hot Springs, Arkansas, reported that of a total of 60 patients with chronic rheumatoid arthritis and treated with testosterone propionate, 46 (76%) responded with definite objective improvement. He also reported on five patients with degenerative joint disease treated similarly. Three were objectively improved. One of four patients with primary fibrositis was improved, whereas none of three patients with gout were benefited. Two patients with the shoulder-hand syndrome were objectively improved.

Dr. R. K. W. Kuipers, specialist for rheumatic diseases at The Hague, Holland, administered testosterone to 20 patients with rheumatoid arthritis.[14] Thirteen or 65% were sufficiently improved to return to previous occupation. In his series, improvement occurred an average of 32 days after beginning of treatment.

In a series of 28 patients, Ashley[15] obtained beneficial results in all but six following the administration of testosterone propionate. This series included a case of Still's disease, one patient with gout, one case of Reiter's syndrome, and two with shoulder-hand syndrome. Ashley concludes "the effects of testosterone appear to be non-specific and applicable to various types of rheumatoid conditions."

Davison, Koets, Snow and Gabrielson[16, 17] were the first to report on the beneficial effects of pregnenolone in rheumatoid arthritis. Gil and Mont[18] administered intramuscularly 100 mg. of pregnenolone per day for a week and followed this with 300 mg. daily of the same substance by mouth for one month. Twenty-two of the 30 patients treated in this manner responded favorably. Since the original report, conflicting opinions have appeared[19, 20] regarding the effects of pregnenolone.

In our first report in 1949,[9] remission, lasting from 4 to 12 weeks,

was induced in 81 of the 90 patients. This included the response to testosterone, pregnenolone, or the two used in combination. The patients were started on either testosterone or pregnenolone. If the compound proved ineffective or if it was found advisable not to continue its use, the patient received the other steroid. If the response to either compound was unsatisfactory, a combination of both was usually administered.

The status of these 90 patients after one year is represented by Series A in Table I. Eighty-six had received a trial course of treatment with pregnenolone and at the end of the year, 18 patients (21%) remained in remission and continued receiving the substance. During this time, 60 of the 90 patients received testosterone and 24 (40%) maintained a favorable response. The remaining 36 (60%), who were designated as negative or failures, include 16 women whose virilizing changes required

TABLE I

	Pregnenolone			*Testosterone Propionate*		
Series	*No.*	*Pos.*	*Neg.*	*No.*	*Pos.*	*Neg.*
A (90 pts.)	86	18	68	60	24	36
B (100 pts.)	69	14	55	64	29	35

cessation of treatment. Their arthritis, however, was responding satisfactorily at the time treatment was stopped. Nine patients maintained improvement on a combination of these two steroids. Estrogens were used originally, but general use of these substances was later abandoned.

In Series B of 100 patients (Table I), pregnenolone was administered orally or by injection of the acetate ester in oil or in aqueous suspension to 69 patients. Of these, 34 (49%) exhibited relief from pain, swelling, and general malaise. It is quite important to note, however, that of those responding favorably, 20 relapsed some time after the third week, usually between the sixth and eighth week. Surprisingly, in five of the patients the substance had to be discontinued because of undesirable side-effects. These reactions were not severe and included such manifestations as dizziness, painful breasts, vasomotor instability, and skin eruptions.

Testosterone propionate was administered to 64 of the 100 patients in Series B (Table I). Twenty-nine (45%) responded with sustained improvement, both subjectively and objectively. This included 13 women who did not experience virilizing changes. Four of these continued to receive testosterone injections, averaging approximately 200 mg. per month. The other nine women remained in remission without additional therapy.

At this time (six months following the second report) the status of the 190 patients is as follows: Six are deceased, and no recent follow-up record could be obtained on 29. The others have been seen or contacted within the past month. Three of the patients developed natural remissions which could not be attributed to the action of either of the two

steroid substances. The status of the remaining 152 patients may be seen in Table II. Most of these patients received both pregnenolone and testosterone, separately and in combination. Of the 142 patients who received pregnenolone, 37 (26%) are in remission and continue to receive this compound. During this latest period it was necessary to discontinue therapy in only one additional patient because of undesirable side-effects. A total of 124 patients received testosterone propionate, and 71 (57%) are in remission at the present time. This does not include eight women who were responding satisfactorily but in whom the testosterone had to be discontinued because of undesirable side-effects.

TABLE II

Pregnenolone			*Testosterone Propionate*		
No.	*Positive*	*Negative*	*No.*	*Positive*	*Negative*
142	37 (26%)	105 (74%)	124	71 (57%)	53 (43%)

Attention is called to the fact that a number of women whose response was designated as unsatisfactory in the preceding reports are now reclassified with the satisfactory group. This change can be explained by the fact that in many of the women who earlier became virilized on larger doses, therapy was later resumed and as soon as a suitable response was obtained they were maintained with much smaller doses. In this manner they are being held in remission without objectionable virilizing effects.

Fifty-nine patients received the combination of testosterone and pregnenolone. This combination was used for two reasons. In a certain number of patients, the degree of response to pregnenolone was not sufficient to maintain them, and the use of testosterone alone was too virilizing. The response to combined therapy warranted continuation. A smaller group of patients included those who did not respond to either testosterone or pregnenolone used singly, but responded to the simultaneous administration of both substances. At the present time 16 patients are being maintained satisfactorily with this combination. Of the 190 patients, 71 responded to testosterone, 37 to pregnenolone and 16 to the combination. Thus, after 18 months, 124 (65%) have received benefit from these two substances, either singly or in combination.

Discussion

A major problem in the study of rheumatoid arthritis has been the difficulty of evaluating the patient's response to therapy. Natural remissions are difficult to distinguish from those that are therapeutically induced, and many honest mistakes have been made in evaluating a new therapeutic regimen.

Several reasons for the discrepancy in the results reported by different observers following the use of testosterone and pregnenolone may be related to dosage and to the fact that many investigators, including ourselves, were expecting an immediate dramatic response. Lack of immediate

response was often called negative. The results of Fisk, Howard and Fay,[11] Patterson,[13] Kuipers[14] and Ashley,[15] as well as our own experiences, have shown that the beneficial effects from these steroids develop slowly.

The dosage requirement for each patient is extremely variable and for optimal results must be individualized. Overdosage may lead to edema, headaches, tinnitus, sense of ill-being, drowsiness, and frequently an exacerbation of the rheumatic symptoms. At first, we administered large doses of testosterone propionate (100 to 300 mg. per day) in an attempt to obtain a more rapid remission. Since then we have learned, as have other investigators, that it is preferable to administer 75 to 300 mg. per week for optimal results. The beneficial effects appear more slowly but the distressing side-effects are at the same time markedly reduced. On the other hand it appears that our original dosage of pregnenolone (100 to 150 mg. per day) may have been insufficient. In recent months we have increased the dosage (400 to 600 mg. per day) with an increase in the percentage of those responding favorably from this substance.

Unfortunately, we have found no objective laboratory procedures which satisfactorily evaluate the status or the clinical course of the disease. The sedimentation rate has been determined periodically in all patients. Although the general trend in those patients who have shown favorable responses to treatment is toward a decrease, we find marked variations which are not well correlated with the clinical picture.

Changes in blood protein relationships may constitute a more dependable indicator of improvement; particularly the return of the albumin and globulin levels toward normal, with an increase in hemoglobin. The urinary output of 17-ketosteroids increases during testosterone administration but is unaltered following pregnenolone. Urinary 11-oxycorticoids are unchanged with either pregnenolone or testosterone. Changes in CO_2 combining power, and carbohydrate metabolism, are not demonstrable. As one would expect from previous work with testosterone, there is a retention of sodium and potassium. Furthermore, positive nitrogen, calcium and phosphorus balances result. When a physician considers prolonged therapy with a new substance for the treatment of chronic disease, these general metabolic effects become important.

Ultimately the most reliable criterion in evaluating the therapeutic response of a patient with rheumatoid arthritis is the test of time. The status of the patient after a year of therapy is more important than his immediate response.

To minimize mistakes in the evaluation of the therapeutic response, we have taken the following precautions:

1. Prolonged observation of the patient (one year or more).
2. Independent opinions of several observers on each patient.
3. Use of placebos.
4. The use of ACTH and cortisone as a "yard-stick" of response to determine the adequacy of the criteria used in evaluating testosterone and pregnenolone therapy.

A group of 26 patients were treated with cortisone or ACTH. The same criteria that we used throughout were applied in assaying the clinical course of these patients. Our results were as follows: Of the 26 patients, 20 (77%) exhibited an immediate beneficial response. During the following six to eighteen months, however, therapy was discontinued in all but nine of these patients, either because of undesirable effects or because they were not held in remission.

We do not believe that pregnenolone or testosterone exerts a specific action against the inflammatory joint lesion, but that they are non-specific, exerting their beneficial effects through generalized metabolic influences. We are continuing our efforts to investigate other substances exerting favorable metabolic effects, without undesirable side-effects.

Conclusions

A total of 124 patients with rheumatoid arthritis have been treated with testosterone propionate for as long as 18 months. The administration of this steroid has resulted in remissions, which were maintained in 71 (57%) of these patients. This figure does not include those in whom the response was favorable but where therapy was discontinued because of undesirable side effects.

Pregnenolone was administered to a total of 142 patients for a period of time ranging up to 18 months. Thirty-seven of these patients, or 26%, maintained a satisfactory improvement through this period of time.

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Discussion

Discussion on this paper follows Chapter 14.

12

Pregnenolone Administration in Rheumatoid Arthritis*

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This report presents results of studies of the clinical effects of pregnenolone in rheumatoid arthritis and spondylitis. The group includes patients seen in the arthritis clinic and medical wards of the Stanford University Hospital, private patients of the author, and a few from the wards of the Letterman General Hospital, San Francisco. There is included but one of the patients previously reported by the author; this individual has received pregnenolone by mouth and injections continuously for 16 months. Pregnenolone has also been administered to patients with discoid lupus, disseminated lupus, asthma, and women with hirsutism. An additional number of patients received the drug for periods too short to make evaluation possible. Six patients, all women, received intramuscular injections for less than one week and pregnenolone was discontinued because of painful nodules at the site of injections.

The classification proposed by Steinbrocker[1] for evaluation of therapeutic agents is used in this report. Only one patient falls into Stage I classification. Since roentgenograms were made in all patients no dispute can arise regarding the validity of diagnosis in the remaining cases. All patients showed evidences of active disease when pregnenolone was instituted. The classification of improvement adheres strictly to the Steinbrocker criteria. Criteria based on change in objective findings serve a useful purpose, but to record only the objective changes and measure improvement only in terms of these changes does not give a complete picture of the patients' response. Subjective symptoms are difficult to evaluate but their relief often indicates real improvement even though they cannot be accurately measured. The relief of pain, fatigue, and stiffness is important to the patient and increases his capacity for work.

This paper does not attempt to prove pregnenolone is a cure for arthritis. It is not. The results reported do show pregnenolone influences favorably the course of the disease in many patients and appears totally

* This work has been supported in part by a grant from the Schering Corporation, Bloomfield, New Jersey. Pregnenolone has been supplied in generous amounts by the Schering Corporation, the National Drug Company, and Wyeth, Inc.

ineffective in others. An attempt will be made to determine if the factors of age, sex, and in women the relation of ovarian function, stage and degree of activity, the mode of administration of the drug, dosage, and time, play a part in determining the result.

The factor of dosage appears to play a significant role in response. In early studies pregnenolone in dosage of 150 mg. daily appeared effective. Subsequently when failures with this dosage were demonstrated, larger doses were tried with significant improvement in the response rate.

Pregnenolone in common with other steroids has some anesthetic effect but this effect is weak in comparison to the anesthetic effect of other steroids. The anesthetic effect may account in part for resulting change seen in these patients but undoubtedly is only one of several effects which the steroid manifests.

One of the difficulties encountered in any study of the effects of pregnenolone in man is lack of ability to measure it in terms of known disciplines. Inability to measure its effects may reflect inadequacy of suitable methods. Sterols in the fecal excretion make it difficult to determine if orally administered pregnenolone is absorbed or excreted unchanged. We have not been able to recover pregnenolone from the urine of patients given as much as 900 mg. daily.

In the report of the treated patients which follows, division is made into patients having uncomplicated spondylarthritis and those with rheumatoid arthritis. The rheumatoid arthritis cases are further segregated into men, women who received the drug by oral administration only, and women who received it by injection or by both the oral and intramuscular routes.

In all of the tables the abbreviations for different forms of pregnenolone administered are as follows:

AA Aqueous suspension pregnenolone acetate intramuscularly.
AC Aqueous suspension crystalline pregnenolone.
AO Pregnenolone acetate in sesame oil or sesame oil-benzyl alcohol.
Or Oral pregnenolone or its acetate.
Acet. 21-acetoxypregnenolone.*

Table I shows the results of pregnenolone administration in spondylarthritis. In early cases of spondylarthritis where limitation of movement is the result only of the inflammatory process and the associated muscle spasm, objective measurement of improvement is possible. When extensive ankylosis is present, objective improvement may be very slight though at this stage the patient may still have active disease. It then becomes necessary to assess results in terms of subjective complaints. Sedimentation rates do not reflect accurately activity in this disease. One of the failures reported in this group has never had a sedimentation rate above 7 mm. in one hour. Furthermore this patient has shown no loss of spine mobility and little muscle spasm. The patient does have extreme fatigue, pain which causes loss of sleep and positive evidence shown by serial roent-

* 21-Acetoxypregnenolone, although termed a pregnenolone by many authors, is actually more closely related to desoxycorticosterone (Ed.).

genograms of progressive disease. His course has been uninfluenced by pregnenolone, 17-hydroxy 11-dehydrocorticosterone (Merck Cortone) and pituitary adrenocorticotrophic hormone (Armour).

W.S. was benefited temporarily by roentgenotherapy in 1947 and 1948. He returned in 1950 with progressively increasing disability, because of pain, stiffness and greater loss of spine mobility which prevented him from putting on his shoes. There was measurable improvement in spine mobility with pregnenolone which made it possible for him again to put on and lace his own shoes, but little if any lessening of pain.

J.D., a young woman of twenty, began to have low back pain in February 1949. She became acutely ill in June with fever and such severe pain in the neck hospitalization became necessary. Roentgenograms showed the typical changes of spondylarthritis in the sacroiliac joints and in the lumbar and cervical segments of the spine. She has received pregnenolone for sixteen months; and for several months has had practically no disability and has worked in shifts as an elevator operator. During this time careful records of menstruation show no change in this function, and the blood cholesterol remains unchanged at 155 mg. %.

Present studies confirm previously reported reduction of high ketosteroid excretion in spondylarthritics coincident with an improved clinical status during pregnenolone therapy. Attention is called to the fact that the figures for ketosteroid excretion reported herewith represent average excretion in 24 hours based on determination of the total crude neutral fraction on a 3-day sample of urine, using androsterone as the standard, and not ketonic fractions.

Seven of the eleven men in the rheumatoid-arthritis group (Table II) who received pregnenolone manifested major improvement. The dosage range in these cases varies from 300 mg. pregnenolone acetate orally to 900 mg. per day. Two of the patients, J.D. and C.de M., returned to their previous states soon after pregnenolone was discontinued and placebo tablets were substituted. Adequate discussion of all cases is impossible in this review but amplification of the brief clinical notes in the cases of B.T. and C.W. is given so the reader will obtain a better picture of the type of patient treated.

B.T. had active spondylarthritis in 1945 and was treated at that time with roentgenotherapy. On this admission to the clinic there was involvement of both feet, both wrists and the left elbow. The joints presented increased skin temperature, swelling, tenderness and loss of mobility; only 10° painful movement was possible in the elbow. He was treated as an outpatient after an observation period of ten days during which time he became worse. At the end of three weeks treatment with pregnenolone by injection there remained only little tenderness in the elbow and full range of motion was possible; the wrists and one ankle remained slightly swollen. Pain and stiffness were absent. By the end of eight weeks all signs of symptoms had disappeared and dosage was gradually reduced while the patient increased his activity. At present he is engaged in full activity on his farm.

TABLE I

SPONDYLARTHRITIS

Case	Age	Sex	Yrs. Duration	Involvement	ESR 1	ESR 2	17 KS	Pregnenolone, Dosage and No. mg./day	Days	Total Gms.	Clinical Notes on Result
D.A.	16	F.	1	S.I.	24.5	25	6.1	OR 300 2/13/50–3/4/50	19	5.7	Symptoms, signs O. Exacerbation when treatment stopped. ESR 25, 10/16/50.
							10.1	OR 300 3/13–3/27/50	15		
								AC } 4/3–5/29/50	56		
								or AO } 50—150 mg./d		9.3	
N.C.	38	M.	6	L.D. Ank.			24.8	AC 600 2/15/50–2/25/50	10	6.0	Symptomatic improvement.
J.D.	20	F.	3 mos.	S.I.	49	40	11.2	AC 100 7/20/49–8/5/49	16		Less pain and tenderness. Spasm controlled. Gained weight. Sleeping well. On this dosage, had recurrence of activity.
				C.L.			7/16/49	AC 150 8/6/49–8/10/49	5		
							19.7	AC 200 8/11/49–8/18/49	8		
							11/21/49	AO 100 8/19/49–10/6/49 Q2D	47		
								AO 150 10/7/49–11/22/49	46		
								AC 150 } 11/23/49–1/14/50 Q2D	26		
								OR 300 }	52		
								AO 150 1/15/50–2/10/50 Q2D	12		
								OR 400 2/14/50–4/4/50	49	56.35	Medication discontinued. Exacerbation after three weeks.
								AO 150 5/9/50–7/6/50	58		
								OR 300 7/6/50–9/25/50	51		
								AO 250 9/25/50–11/13/50 3 × week	14		
								OR 300 11/20/50—present	39	39.2	
K.G.	25	F.	7	S.I.	10		20.5	AO 100	14		Low backache only, completely relieved in two weeks. Tenderness persisted. 24th day, ESR up to 17. Eos. increased from 3 to 40.
								AO 150	10		

L.L.	42	M.	1	C.L.	46	50	14.0	AC	300	1/9/50–2/3/50	25		Loss of stiffness in neck and lower back. Gradual improvement, beginning in two weeks. Symptomatically free in two months.
							25.9	AO	200	2/4/50–2/8/50	5		
								OR	300	2/9/50–2/16/50	8		
								AO	300		8		
								OR	600	2/17/50–2/24/50	8		
								OR	900	2/25/50–3/8/50	14		
								AO	100	3/6/50–3/8/50	3		
								OR	300		3	31.9	Injections Q 2 day + oral daily after dates given. Report 10/1/50 symptom-free.
C.L.P.	35	M.	7	C.D.L.			24.8	AC	100	1/26/50–2/14/50	20		Much pain and muscle spasm relieved by pregnenolone; 17 KS fell on therapy.
								AC	200	2/15/50–2/28/50	14		
								AC	100	3/1/50–3/7/50	7	5.5	
W.S.	43	M.	5	C.D.L.	33	44	9.2	AO	200	6/1/50–6/11/50	11		Greatly increased mobility; pain persisted. Nodules in buttocks from injections.
							15.6	AC	400	6/12/50–6/21/50	12		
								AC	300	6/22/50–7/5/50	14		
								AC	300	7/6/50–8/18/50	44		
								OR	300		44	37.6	
V.T.	38	F.	4	S.I.	15	69	27.6	AO	100	9/18/49–1/6/50	111		Little disability but nagging pain prior to R. Improved; not completely free. Exacerbation 2/13/50. Response to AO better than AC. Continues symptomatically free on AO.
				L.			23.9	AC	150	1/7/50–1/28/50	22		
								AC	200	2/14/50–4/6/50 Q2D	25		
								OR	600		50		
								AO	200	4/7/50–6/20/50	74		
								AO	150	6/21/50 to present 3 × week		76.9	

KEY TO ABBREVIATIONS: Involvement: S.I., Sacroiliac joints; L., Lumbar; D., Dorsal; C., Cervical; Ank., Ankylosed. ESR 1, At beginning of treatment. ESR 2, At end of treatment. OR, Oral pregnenolone. AC, Aqueous suspension. AO, Pregnenolone in sesame oil. AA, Aqueous acetate. Acet., 21-acetoxypregnenolone. KS, Ketosteroids.

Table II
Rheumatoid Arthritis, Men

Case	Age	Yrs. Duration	Stage	Function	Improvement	ESR 1	ESR 2	Pregnenolone, Dosage and No. mg./day	Days	Total Gms.	Clinical Notes on Result
A.M.	48	9	II	II	II	35	39	OR 300 6/19/50—present	154	46.2	Much improvement. Good result.
G.B.	26	2	I	II	IV		39	OR 600 9/15–10/12/50	27		
								OR 400 10/13–10/20/50	7	19.0	No benefit.
W.M.	67	4	II	III	II	31	40	AO 150 5/9–6/5/50	28		
								AO 200 6/6–6/12/50	13		
								AO 150 6/13–7/28/50 Q2D	22		
								OR 300 8/3–8/21/50	19		Exacerbation on 300/d dosage.
								AO 150 8/21–9/18/50 Q2D	13		
								OR 600 9/19–11/29/50	72	60.95	Steady improvement. Continues symptom-free.
B.P.	60	8	IV	III	II	36		AO 200 3/2–3/24/50	23		Exacerbation, then improvement in 2 weeks after B begun.
								AO 100 3/25–5/11/50	47		
								OR 300 3/25–4/10/50	17		
								AO 100 5/12–7/6/50 3 × weekly	24	16.8	Exacerbation.
B.T.	38	14	II	III	II	9	8	AC 400 6/12–7/10/50	30		6/19 much improvement. 7/10 does very well.
								AC 300 7/11–8/7/50	27		
								AC 300 8/8–9/11/50 3 × weekly	15		
								AC 300 9/12—present 2 × weekly	5	26.4	Complete remission.

N.W.	39	3	II	III	III			AA	300	1/26–2/2/50	8		
								AO	200	2/3–2/16/50	14		
								AO	100	2/17–3/16/50	30		
								OR	900	2/17–3/27/50	37		3/27 no change.
								OR	450	3/28–4/27/50	31		4/27 improvement.
									none				5/11 same.
J.D.	48	18	IV	III	III	43	2	OR	300	11/28–12/12/49	15		12/12 stiffness less.
								OR	600	12/13–12/19/49	6		
								OR	300	12/30–12/30/49	11		No tablets on 12/31.
								OR	300	1/6–3/19/50	72		Improved slightly.
									Placebo	3/20–4/17/50			Reactivation when pregnenolone stopped.
									Then, no Ꝑ available				
								OR	300	6/5–7/3/50	28		
								OR	600	7/4–7/25/50	22	54.6	No response.
C.W.	45	3	III	III	II	34	36	AO	300	3/17–4/20/50	34		
								AC	300	5/15–5/19/50	15		See text for discussion.
								AC	200	5/19–5/25/50	7		
								AO	150	5/26–6/9/50	15	18.35	
D.Y.	33	10	IV	III	II	47	21	AC	300	3/31–4/28/50	30		Improvement began
							(4/20)	AC	300	5/12–5/17/50	6		4/4/50. 1st period hospi-
								AC	300	5/26–6/16/50	23		talized. Subsequent treat-
							9	AC	300	6/23–6/30/50	8		ment as out-patient.
							(6/30)	AC	300	7/7–7/14/50	8		
								AC	200	7/15–8/4/50	20		
								AC	300	8/11–9/1/50	22		
								AC	300	12/8—present	20	37.1	11/9/50 still improving.

TABLE II—*(Continued)*

RHEUMATOID ARTHRITIS, MEN

Case	*Age*	*Yrs. Duration*	*Stage*	*Function*	*Improvement*	*ESR 1*	*ESR 2*	*Pregnenolone, Dosage and No. mg./day*	*Days*	*Total Gms.*	*Clinical Notes on Result*
C.deM.	56	5	III	IV	II		27	OR 300 2/27–3/20/50	22		Rapid improvement 1 week. All joint swelling gone 3 weeks. No change on placebo 20 days, then rapid return to pre-treatment status. Improved again on AO. 11/20 no signs of rheumatic activity. 12/11 return to complete disability.
								Placebo 3/20–5/15/50			
								AO 200 5/15–5/29/50	15		
								AO 100 5/29–7/15/50	47		
								OR 600 10/2–11/20/50	48	43.1	
M.G.	30	9	IV	IV	III	40	46	AC 400 7/14–7/19/50	5		Subsequent Cortone for 10 days without improvement.
								AC 400 7/24–8/2/50	10	6.0	
L.H.	59	1½	IV	IV	IV	57	56	AC 300 2/16–2/28/50	13		Early improvement not sustained.
								AC 300 3/6–4/3	29		
								AC 150 4/5–4/16/50	12	14.4	
M.S.	37	8	III	IV	III			AC 300 2/1–2/14/50	14		Improvement. Exacerbation without Ꝑ. Improvement, then subjectively worse. Ꝑ begun without patient's knowledge. Improvement.
								AC 600 2/15–2/24/50	10		
								AC 450 3/1–4/5/50	36		
								Placebo 4/6–4/20/50			
								AC 4/20–5/8/50		26.4	

C.W. has severe rheumatoid arthritis and pulmonary tuberculosis. The tuberculosis is being treated by pneumothorax. Gold sodium thiosulfate for ten weeks in 1949 induced partial remission. When first seen mobility of the right elbow was much impaired; it was swollen, hot, tender and the capsule was distended. Both shoulders were painful and tender, both knees were hot and contained much fluid. 100 milligrams of pregnenolone acetate in 75% sesame oil–25% benzyl alcohol were given every eight hours intramuscularly by the patient's wife, a trained nurse.

For the first week he felt worse and then suddenly noticed great improvement in the shoulders and feet followed by complete disappearance of pain, heat and swelling from the left knee. The right knee became less stiff but still contained some fluid March 30, 1950. For the next two weeks two injections, each 150 milligrams, were given daily. On April 20th pregnenolone was stopped because of abscess in both buttocks. Cultures from these abscesses showed staphylococci. Pregnenolone in aqueous suspension was begun May 5th and he again improved. In fourteen days he was able to get up easily from a low chair, previously impossible since the beginning of his illness. Painful nodules developed from injections and the medication was discontinued.

The failures in cases M.G. and L.H. can only be explained by the fact they had advanced disease. M.G. improved no more on ten days treatment with Cortone (Merck) than he did on the fifteen-day trial of pregnenolone. The extent of disability in such cases makes evaluation of any therapeutic agent exceedingly difficult.

Eleven women who received oral pregnenolone only are listed in Table III. Most of these patients fall in the older age group. All of the patients who failed to obtain material benefit from pregnenolone were either postmenopausal or in the menopause, with the exception of M.S., a young woman of 29 who had much irregularity in menstruation and severe dysmenorrhea. She showed definite symptomatic improvement during the 2 weeks she received 600 mg. pregnenolone daily. During that time she had the first painless period she could remember. Unfortunately we were unable to continue the medication because of supply shortage, and a subsequent trial of pregnenolone, 300 mg. daily for 2 weeks was without benefit. I.J. has had severe arthritis for years with considerable destruction of the hip joints and large effusions in both knees. Dosage of pregnenolone in this case and length of time of administration were both adequate but improvement was slight and not sustained.

B.A., a 19-year-old girl had great fatigue, and painful inflamed joints. She had been helped by chrysotherapy though she had never gone into remission during the 4½ years of her illness—she took from 30 to 40 gms. of acetylsalicylic acid daily for relief of pain and stiffness—she stopped the analgesic at the end of the first week on pregnenolone. By the end of the third week, stiffness, fatigue, and tenderness were gone and she was able to go shopping, to her a great achievement. After 5 days on pregnenolone menstruation began, the first painless period she had ever experienced. December 5, 1949, daily dosage of pregnenolone was increased

TABLE III

RHEUMATOID ARTHRITIS, WOMEN
ORAL ONLY*

Case	Age	Yrs. Duration	Stage	Function	Improvement	ESR 1	ESR 2	Pregnenolone, Dosage and No. mg./day	Days	Total Gms.	Clinical Notes on Result
B.A.	19	4½	IV	II	I			300 11/21–11/28/49	8		Improvement in one week.
								400 11/29–12/5/49	8		Complete relief. All stiff-
								600 12/5–1/4/50	30		ness and fatigue gone by
								300 1/19–3/27/50	67	43.7	12/15/49. Medication dis-
											continued because of pro-
											longed menstruation
											1/4/50.
E.C.	50	20	III	II	II	56		600 3/27–4/16/50	20		Improvement noted on
								600 4/20–4/27/50	8	16.8	4/4.
								Placebo 4/27–6/5/50			6/5 doing quite well.
I.J.	43	13	III	II	IV		42	600 12/9/49–1/18/50	40		1/6 some improvement.
								300 1/19–2/16/50	28		3/7 left knee painful,
								900 2/17–3/28/50	39	67.5	with fluid.
											3/28 no sustained im-
											provement.
V.M.	44	22	IV	II	II	20	30	300 2/2–2/9/50	7		
								900 2/9–2/23/50	15		
								300 3/7–5/5/50	60		
								200 5/6–7/28/50	83		Soreness and stiffness de-
								(AC 200 7/28–8/31/50) 3 × weekly	15		creased. Nodules from in-
								500 9/1–10/25/50	55		jections.
								300 10/26–11/26/50	31	97.3	Much improved.
I.O.	47		III	II	IV			300 1/10–1/31/50	21		Irregularity in menses not
											altered by ℞.
											No improvement.

M.S.	29	3	II	II	IV	26		600 3/13–3/27/50	14		
								300 4/10–4/17/50	8		
								300 6/2–6/16/50	10	13.8	No benefit.
E.C.	48	12	IV	II	IV	40	43	600 1/13–2/10/50	29		
D.S.								900 2/11–2/23/50	12	25.8	Previous temporary benefit from chrysotherapy; subsequent injection of 400 mg. 21-acetoxypregnenolone for 15 days 4/15–4/29 without improvement.
M.C.	61	2	III	III	IV	21	36	300 11/25–12/21/49	28		
								600 12/22/49–1/6/50	16		1/6 some improvement.
								300 1/7–2/6/50	31		
								600 2/7–2/13/50	7		
								900 2/14–3/1/50	15	45.0	On 5th day of 900, temporary improvement, then exacerbation.
V.L.	66	5	III	III	II	51		300 1/20–2/3/50	14		Some improvement with 300 dosage. Completely free of joint swelling and tenderness on 900 mg. Reactivation 2 weeks after ℞ discontinued.
								600 2/4–2/10/50	7		
								900 2/11–3/7/50	25		
								600 3/8–3/20/50	13		
								900 3/21–4/2/50	13	50.4	
I.N.	52	2*	II	III	IV	37	39	600 12/12–12/28/49	16		Psoriatic. Subjective improvement slight and temporary.
								800 1/28–2/21/50	25		
M.U.	65	13	IV	III	IV	35		900 2/24–3/9/50	14		Some improvement.
								600 3/10–3/23/50	14		
								300 3/24–3/31/50	8		No sustained benefit.

* Except for period of intramuscular injections in the case of V.M., all therapy was by oral route.

to 600 mg. and continued until January 4, 1950. It was discontinued at that time because of its possible relationship to prolonged menstrual bleeding that began December 22, 1949 at normal time interval but which continued until January 9, 1950. On dosage of 300 mg. daily for 67 days beginning January 19th the patient continued free of all objective evidence of arthritis activity and without any aberration of menstruation.

In Table IV is summarized the experience of 30 women who received pregnenolone by intramuscular injections or intramuscular injections supplemented by oral administration. Daily dosage, the period of treatment and results are given for each individual patient; one patient with grade I functional impairment was benefited promptly. The rapidity of the change appears to rule out a favorable effect of the pregnancy, which was then in its fourth month during which time the patient had become steadily worse though 2 previous pregnancies had induced temporary remissions. The factor or factors by which pregnancy induces remission are not yet known. Since corticosteroid excretion is observed early, this may be a factor. On the other hand, corticosteroids fall to normal before parturition, yet postpartum blood appears to be an effective therapeutic agent. The pregnanediol excretion of this patient February 11, 1950 was 2.4 mg./24 hours; coincident with her clinical improvement following pregnenolone administration for nine days, it increased to 63.5 mg./24 hours.

Five patients with grade II functional impairment were given pregnenolone as shown in the table. One of these patients, M.S., showed no objective change; the periods of treatment and dosage may be the factors responsible for failure. D.W. who was completely relieved of symptoms and signs of active rheumatic disease became psychotic at the fourteenth day of treatment and medication was discontinued. She had had previous psychotic episodes.

In the group of 18 patients having grade III functional impairment, 50% show a grade II or better response (E.M.F. to L.W.). The clinical notes on the individual patients are self explanatory.

Some of the patients, A.M.J. and L.H., showed great improvement initially but failed to maintain the improved status and so are classified as grade IV response. (M.B., E.J. and B.S. received too small doses of the drug.)

Review of the literature shows many attempts to compare pregnenolone to 17-hydroxy-11-dehydrocorticosterone. The two substances are very unlike and the writer is not at all convinced that pregnenolone acts by conversion to a steroid similar to cortisone.

Some of the unfavorable reports based on one case using dosage similar to an effective dose of cortisone presume failure before the experiment is begun.

Dosage of pregnenolone varies as with other steroid hormones. The present report shows it does influence favorably the course of rheumatoid arthritis. It produces no toxic effects even when it is given for long periods of time.

TABLE IV

RHEUMATOID ARTHRITIS, WOMEN

Case	Age	Yrs. Duration	Stage	Function	Improvement	ESR 1	ESR 2	Pregnenolone, Dosage and No. mg./day	Days	Total Gms.	Clinical Notes on Result
J.K.	28	12	II	I	II	43		AO 200 } 2/15/50–3/4/50	18		Patient pregnant 4 mos. Low pregnanediol excretion, increased after 1 week's treatment.
								OR 600 } 2/15/50–3/4/50	18		
								AO 100 3/5/50–3/11/50	7		
								AO 100 } 3/12/50–3/18/50	7		
								OR 400 } 3/12/50–3/18/50	7	9.3	
D.B.	58	2	II	II	II	38	39	AC 300 4/1/50–4/19/50	19		Increased mobility and decreased swelling and tenderness within 5 days. Exacerbation of symptoms 10 days. At end of 6 weeks ESR unchanged. Acetoxypregnenolone 5/29–6/9. Patient regressed. Nodules from acetoxy.
								AC 200 4/20/50–4/25/50	6		
								AC 300 4/26/50–5/5/50	10		
								In May, 200–150 daily injection			
								AO 150 5/16/50–5/26/50	11		
								AO 300 5/29/50–6/9/50	11	14.1	
E.H.	67	17	IV	II	II	26	31	AC 400 6/21/50–7/5/50	15		Subjective and objective improvement began in 10 days. Daily fever on admission to hospital, subsided. Injections discontinued because of local tissue reaction.
								AC 300 7/6–7–8–10–11	5		
								OR 400 7/6/50–7/21/50	16		
								OR 600–300–400–900 July through November	153	105.7	
A.LaC.	63		II	II	II	35	50	AO 200 5/12/50–5/26/50	15		Free from pain and stiffness at end of 2nd week. Able to do housework.
								AO 100 5/27/50–5/31/50	5		
								AO 100 6/1/50—Q2D			

TABLE IV—*(Continued)*

RHEUMATOID ARTHRITIS, WOMEN

Case	*Age*	*Yrs. Duration*	*Stage*	*Function*	*Improvement*	*ESR 1*	*ESR 2*	*Pregnenolone, Dosage and No. mg./day*	*Days*	*Total Gms.*	*Clinical Notes on Result*
M.S.	29	3	II	II	IV	26		AO 600 3/13/50–3/27/50	15		Improved subjectively. No objective change.
								AO 300 4/10/50–4/17/50	8		
								AO 300 6/2/50–6/16/50	15	15.9	
D.W.	35	8	III	II	II	10		AO 200 2/11/50–2/23/50	13	2.6	Psychotic episode. Treatment discontinued.
E.M.F.	58	12	III	III	III	39.5	29	AC 300 8/21/50–9/25/50	36	10.8	Continuous pain completely relieved by 14 days. Increased mobility and diminished signs of activity.
E.B.	29	12	IV	III	II	51		AC 300 } 4/21/50–4/24/50	4		Previous relief during pregnancies. Much pain and joint swelling, hips, knees and feet. Unable to walk. Shoulders and wrists bad. Pain gone after 3 days. Walking without pain, 30 days. 11/6/50, limited only by irreversible changes.
								OR 300 }	4		
								AC 300 4/25/50–5/22/50	27		
								AC 150 5/23/50–6/12/50	20		
								AC 100 6/13/50–7/10/50	27		
								AC 600 weekly	122	26.4	
L.B.	52	1	III	III	II	53	49	AC 200 2/13/50–3/30/50	45	9.0	Acute onset; hot, swollen knees, ankles, hands and shoulders; unable to bathe or comb hair. Complete disappearance of all physical signs of activity.

M.B.	50	6	III	III	IV			AC 400 3/24/50–4/22/50			12.0	Pain free in two weeks, some joint tenderness remaining.
								AC 150 OR 300 } 4/23–6/8/50 Q2D			3.6	Became worse on this regimen but tired of injections; menopausal.
V.D.	54	2½	I	III	II	37		AC 300 11/10–12/10/49 AO 300 12/17–1/3/49 OR 600 3/11/50–4/11/50	31 18		33.3	First improvement 12 days. Swelling of hands gone 3 weeks; some residual stiffness. 12/29 acute allergic reaction. Nodules from injections.
M.E.	45	5	III	III	IV	49	38	OR 600 3/16–4/9/50 AO 150 OR 300 } 5/5–5/25/50	23 21		23.25	Menopausal. Complicated by degenerative joint disease in fingers. No improvement.
H.F.	70	22	IV	III	II	47	46	AC 400 } 5/4–5/11/50 200 } 5/12–5/23/50	8 11		4.8	Arteriosclerosis. Ankylosed joints. Much pain and stiffness completely relieved. Refused further injections.
F.H.	53	7	IV	III	IV	33		AO 200 10/4/49–10/20/49 AO 100 10/21–11/2/49 None 11/3–11/15 AC 150 11/15–12/1/50 AC 300 12/2–12/19	16 12 15 17		11.1	Sudden change, increased strength and joint mobility 5 days. Worse on AC. Initial improvement followed by leveling off.
E.J.	55	7	III	III	IV	61	34	AC 300 3/12–4/11/50	30		9.0	No improvement with injections daily; complained of pain with each injection.

TABLE IV—*(Continued)*

RHEUMATOID ARTHRITIS, WOMEN

Case	*Age*	*Yrs. Duration*	*Stage*	*Function*	*Improvement*	*ESR 1*	*ESR 2*	*Pregnenolone, Dosage and No. mg./day*	*Days*	*Total Gms.*	*Clinical Notes on Result*
A.M.J.	37	6	IV	III	IV			Placebo 5 days			
								AC 450 3/1–3/20/50	20	9.0	Remarkable improvement; loss of pain, increased mobility and general well-being.
								AO 100 OR 300 } 3/21–3/27/50	7		3/27, flare-up.
								AO 100 OR 500 } 3/27–4/10	14		
								AC 300 4/10–4/14	4		Improved again.
								O-A 250 4/15–4/28	14		
								AC 300 4/29–5/26	28		Joint swelling.
								OR 500 5/26–6/15	21	34.8	
G.K.	38	10	III	III	IV	27	27	AC 300 1/26–2/16/50	22		Much improved but not completely controlled.
								AC 300 2/24–2/27	4		
								OR 600 2/28–3/20	21		
								300 3/21–4/17	28		
								None 4/30–6/22			Relapse when pregnenolone stopped.
								AC 150 6/23–8/6	43	35.25	
E.L.	31	8	IV	III	II/III			AC 300 3/21–4/4/50	15		Inflamed knees with fluid subsided after 13 days.
								AC 450 4/5–4/12	8		
								300 4/13–4/20	8		No further improvement with larger dose.

H.L.	43	2	II	III	II			AO	150	2/3–2/17/50	15		No change. 3/1, swelling gone from hands, knees no longer hot or tender. 3/12 asthma. Some pain at menses. No sign of joint inflammation. No disability. 10/24 (menopausal).
								AO OR	150 600	2/18–3/8	19	16.5	
								AC OR	150 Q2D 600 D	3/15–3/27	13	8.80	
								AC OR	150 Q3D 600 D	4/1–6/30	91	59.1	
L.M.	49	4	III	III	II	56	57	AO	150/200	2/8–2/27/50	20		Nodules.
								OR	900	2/27–3/16	17		All pain and stiffness gone, 1 week. No oral available. Swelling and tenderness in fingers gone. No pain.
								AC	300	3/16–3/30	15		
								AC OR	200 300	3/30–4/14	14	33.8	
B.S.	36	10	II	III	IV	60	49	AC	200	3/23–4/4/50	13		No improvement. Refused further treatment.
								AC	400	4/12–4/19	8		
M.S.	30	6	II	III	II	51	21	AC	200	10/15–11/15/49	30		Fever, anemia, leukocytosis much improved by 10/23. Still some symptoms to 12/23. 1/6/50 all pain, stiffness, tenderness and swelling gone from fingers, hands and wrists. Still some fluid 1 knee; on oral 300 flare-up.
								AO	200	12/12–1/6/50	26		
								OR	300	1/7–3/10/50	63	35.9	
								OR	600	3/11–4/3/50	24	14.4	
												50.3	
L.J.V.	52	16	IV	III	IV	30	48/51	AC	450	6/30–7/20/50	21		Generally better. Rapidly reverted to previous status on one injection 200.
								AC	200	7/21–8/22	33		

TABLE IV—*(Continued)*

RHEUMATOID ARTHRITIS, WOMEN

Case	*Age*	*Yrs. Duration*	*Stage*	*Function*	*Improvement*	*ESR 1*	*ESR 2*	*Pregnenolone, Dosage and No. mg./day*	*Days*	*Total Gms.*	*Clinical Notes on Result*
L.W.	47	5	III	III	II	54		AC 450 4/8–4/14/50	7		4/12 all tenderness and swelling gone.
								AC 320 4/15–4/17	3		4/17 elbow tender again.
								AC 450 4/17–4/30	13	9.9	Complete disappearance all signs of activity. Discontinued because of nodules.
M.C.	31	5	III	IV	II	45	40	AC 300, OR 400 } 3/1–3/12/50	12		Unable to walk or dress. Much pain and stiffness.
								AC 200, OR 600 } 3/13–3/16	4		Improvement began 4th day.
								AC 300, OR 600 } 3/17–3/20	4		By 3rd week walking well, no pain, no tenderness.
								AC 200, OR 300 } 3/21–4/20	31	30.8	Good muscle strength.
T.G.	51	14	IV	IV	IV	38	35	AC 300 11/19–12/8/49	19		Temporary improvement.
								OR 600 12/9–12/23	15	14.7	Injections in hospital: on oral at home. Improvement not maintained.
J.K.G.	8	4	IV	IV	III	37		AO 100 4/15–6/5/50	21		Definite improvement with increased joint motion. Many ankylosed joints.
								OR 150 6/5–6/20	16		

L.H.	49	7	III	IV	IV	42		AO	300	4/19–5/1/50	13		Dramatic temporary improvement 1 week. Patient had fever and leukocytosis 12–16,000. Improvement not sustained.
								AC	200	5/2–5/18	17		
								AC	300	5/19–5/25	6	4.4	
C.McR.	56	11	IV	IV	III	36		AO	300	2/16–2/25/50	10		Pain and stiffness relieved; no improvement in strength or joint mobility. Allergic reaction to solvent.
								AC	300	2/26–3/11	14		
								OR	600	3/7–3/29	22	20.4	
J.Mu.	22	4	IV	IV	III	32	38	AO	200	2/6–3/6/50	28	5.6	Relief of pain. Increased mobility of joints. Less swelling and stiffness. Treatment discontinued because of abnormal vaginal bleeding. Previous amenorrhea.

One of the serious drawbacks to its use by intramuscular injection is its insolubility and the production of foreign body tissue reactions. None of the modifications of aqueous suspension so far tried overcomes this disadvantage.

Further trial of pregnenolone appears justified in patients of younger age groups and less advanced stages of arthritis than the majority of the patients reported in this paper.

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Discussion

Discussion on this paper follows Chapter 14.

13

Oral Steroid Medication in Rheumatoid Arthritis

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The therapeutic efficacy of cortisone in rheumatoid arthritis has stimulated the investigation of other steroids which might be effective in this condition with possibly less untoward effects and with a more permanent therapeutic effect. The Arthritis Clinic at the Worcester Memorial Hospital has served this purpose by testing the effectiveness of several new substances by the oral route. The patients reported in this study all have rheumatoid arthritis by the criteria of the American Rheumatism Association. In order to secure uniformity in description, they were all, after the initial examination, classified by degree of progression of the disease (stages 1–4) and by the amount of functional disability (classes 1–4). After treatment they were then evaluated as to the amount of improvement (stages 1–4). The procedure usually followed was to administer a placebo medication at the initial visit for a two-week period to determine the psychologic effects of the new therapy. If an improvement was noted by the patient, he or she was maintained on the placebo tablets. If none was noted, one of the steroids was administered. Such psychologic effects in our experience are never marked but are sufficiently frequent to make it necessary to be extremely conservative in the evaluation of any new medication. Examples of this fact are the first two studies shown, in which (1) testolactone and a placebo were administered to patients by code numbers so that the identity of the medication was unknown to the investigators, and (2) Δ^4-androstenedione and a placebo were similarly handled.

In the first study (Table I), 11 patients (3 men and 8 women) were given testolactone in doses of 400 mg. daily by mouth for periods up to a month. A similar number of patients (4 men and 7 women) were given placebos for the same time. Most of these patients had been given pregnenolone previously and had responded little (grade 3) or not at all (grade 4). The first two patients took the testolactone less than one week and discontinued it because of digestive disturbances or an aggravation

of the existing symptoms. Most of the others took the medication for four weeks. In some instances the results seemed to be a bit better than with pregnenolone; in others, worse. In only one case (no. 4) was the result a definite improvement. The patients who noted improvement

TABLE I

THERAPEUTIC EFFECTS OF TESTOLACTONE AND PLACEBO MEDICATION ADMINISTERED "BLINDLY" TO PATIENTS WITH RHEUMATOID ARTHRITIS

		Testolactone				
Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	400	3/7	4	3	Felt worse—stopped med.
2	F	400	5/7	4	3	Heartburn—stopped med.
3	F	400	2	3–4	..	Less pain at night
4	F	400	4	2–3	3	Relapse in 2 wks.
5	F	400	4	3	..	Relapse in 2 wks.
6	M	400	4	3	..	More motion
7	F	400	4	3	4	Less pain—relapse in 2 wks.
8	F	400	4	3	4	Less pain—relapse in 2 wks.
9	F	400	4	3–4	4	Slightly less pain
10	M	400	2	4	3	
11	M	400	4	4	4	
		Placebo				
Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
12	F	400	2	3	3	Less pain
13	M	400	4	3	3	Less pain—more motion
14	M	400	4	3	..	Less pain
15	F	400	4	3	..	Less pain—more energy
16	M	400	4	3	..	Less pain—more energy
17	F	400	4	3	3	Less pain and swelling
18	F	400	4	3	3	Less pain and stiffness
19	F	400	4	3	2	Less pain
20	M	400	4	4	4	
21	F	400	4	4	4	Less pain first 2 wks.
22	F	400	4	4	..	

usually stated that the intensity of the pain had decreased. In general, approximately half the patients felt more comfortable during the period of medication. It is of interest that two weeks after discontinuance of the medicatior there was an exacerbation of symptoms in four patients while

TABLE II

THERAPEUTIC EFFECTS OF Δ^4-ANDROSTENEDIONE AND PLACEBO MEDICATION ADMINISTERED "BLINDLY" TO PATIENTS WITH RHEUMATOID ARTHRITIS

		Δ^4-Androstenedione				
Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	1000	3	2–3	3	Definitely improved
2	F	1000	3	3	..	Less pain—more energy
3	F	1000	3	3	..	More motion
4	F	1000	3	3	3	Less pain—more motion
5	M	1000	3	4	..	
6	M	1000	3	4	..	
7	F	1000	3	4	2	
8	M	1000	3	4	3	
9	F	1000	3	4	3	
10	F	1000	4	2–3	3	Marked relief in pain
11	M	1000	4	3	..	More motion
12	F	1000	4	3	3	Less pain—more motion
13	F	1000	4	3	..	More motion
14	M	1000	4	3	4	More motion
15	F	1000	4	3–4	4	More motion
16	F	1000	4	3–4	4	Less pain
17	F	1000	4	4	3	
18	M	1000	4	4	..	
19	M	1000	4	4	..	
20	M	1000	4	4	3	
		Placebo				
Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
21	F	1000	2	4	4	Not as well
22	M	1000	3	3	4	Less pain
23	M	1000	3	3–4	3	Less pain
24	M	1000	3	3–4	3	Slightly more motion
25	M	1000	3	4	4	
26	F	1000	3	4	3	Felt worse
27	F	1000	4	3	..	Less pain and fatigue
28	M	1000	4	3	3	Less pain and fatigue
29	F	1000	4	3	2	Less pain
30	M	1000	4	3	..	More motion
31	F	1000	4	4	..	Nauseated last 2 weeks
32	F	1000	4	4	4	
33	F	1000	4	4	4	
34	F	1000	4	4	2	Worse
35	F	1000	4	4	..	

they were again on placebos. In considering the effects of the unknown placebo medication, the results are quite similar. Eight of the 11 subjects noted less pain in their joints. None showed any striking improvement. It is evident that on this dosage and in this period of time, testolactone is not effective.

A similar study was made with Δ^4-androstenedione and a placebo (Table II). Twenty patients received the steroid by coded numbers in daily doses of 1.0 gm. daily, nine for three weeks and 11 for four weeks. Two patients (nos. 1 and 10) showed a fair degree of improvement;

TABLE III

THERAPEUTIC EFFECTS OF Δ^4-ANDROSTENEDIONE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*
1	M	500	1	4	4
2	M	500	1	3	3
3	M	500	2	4	4
4	F	500	2	3	3
5	M	500	2	4	4
		1000	2	4	4
6	F	500	3	3	3
7	M	500	3	4	4
8	F	500	4	4	3
9	F	1000	1	3	3
10	F	1000	2	4	3
11	F	1000	2	4	4
12	F	1000	2	4	4
		500	2	4	4
13	F	1000	4	3	..

seven others felt some relief from pain with increasing mobility; the other 11 showed little or no change. Fifteen patients received placebos by code: one for two weeks, five for three weeks, and nine for four weeks. Five reported some feeling of improvement, none very striking. It may be concluded, therefore, that Δ^4-androstenedione by mouth in this dosage as administered by us had no therapeutic value.

At this point it may be mentioned that a mild degree of improvement can be regarded as of little value in judging the efficacy of any specific treatment. These studies may be criticized on the grounds that the majority of the patients used for the clinical trials of other steroids had previously failed to respond to pregnenolone therapy, and therefore also might be less likely to respond to other steroids. This is a valid point, since comparison with pregnenolone is desired, and is due partly to the fact that the turnover in the clinic was greater in the patients who had

shown good results with pregnenolone so that there was a greater accumulation of poor responders. However, as we shall see later, a proportion of poor responders to oral pregnenolone respond well to oral cortisone.

Table III shows the results of an earlier study in which 13 patients were given Δ^4-androstenedione by mouth in doses of 0.5 to 1.0 gm. for shorter periods of time. The results were poor, with only five patients

TABLE IV

THERAPEUTIC EFFECTS OF 16-DEHYDROPREGNENOLONE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	M	500	1	4	4	
		600	1	3–4	4	Slightly less pain
2	F	500	1	3–4	4	
		600	2	4	..	
3	F	1000	2	4	4	
4	M	500	2	4	4	
		1000	4	4	4	
5	F	500	2	3	3	
		500	2	4	3	
6	F	500	2	4	3	More swelling and pain
7	M	500	2	4	4	
8	F	600	2	4	2	More swelling and pain
9	F	500	3	3	3	
10	F	500	3	2	2	
11	F	500	3	3	3	Slightly more motion
12	M	500	3	3–4	4	Slight increase in energy
13	F	500	3	3	3	
		600	1	4	3	More stiffness and pain
14	F	500	4	4	4	
15	F	500	4	4	4	
16	F	1000	4	4	3	More swelling
17	M	500	4	3	3	
18	F	500	4	4	4	
19	F	500	6	3	3	Slightly less pain

showing even a mild degree of improvement. The data on grade of improvement are for all practical purposes identical with those of Table II, indicating that the judgment of the investigators is not biased.

16-Dehydropregnenolone (Table IV) was administered to 19 patients in daily dosages of 500 to 1000 mg., usually in the smaller amounts, for periods varying from 1 to 6 weeks. The average period of medication was 2.7 weeks. Only one patient showed a striking degree of improvement (no. 10); a woman who had responded well to pregnenolone. Five patients

showed mild improvement, and the others little or none. The results are therefore essentially negative.

Dehydroisoandrosterone acetate (Table V) was given to eight patients in daily dosages of 200 to 500 mg. for two to seven weeks. Again, the results were largely negative. A mild acneform eruption was noted in one woman.

Thirty patients were given Marisone (Ayerst) (Table VI) in daily dosages varying from 1000 to 2000 mg. for periods varying from one to eight weeks. The average course was four weeks. The results were poor.

TABLE V

THERAPEUTIC EFFECTS OF DEHYDROISOANDROSTERONE ACETATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	200	2	4	3	No improvement
2	F	200	3	4	4	"Pimples" on face More pain
3	F	200	2	3–4	4	Less pain
		500	7	3	4	Less pain
		500	2	4	4	
4	M	300	4	4	3	More swelling
5	F	500	2	4	4	
6	M	500	2	4	4	
7	F	500	2	4	3	Worse pain
8	F	500	5	3	3	

No patient showed a striking improvement. In 11 instances there was a mild improvement. Four patients were nauseated either from the medication or the gelatin capsules in which it was enclosed. Three older patients had brief episodes of vaginal bleeding; one younger woman missed a period. It is evident that the drug had some estrogenic activity.

Δ^4-Pregnenetriol-3,17,21,one-20 (Table VII) was administered to nine patients, three of whom had responded well to pregnenolone. The responses were poor although in this case, owing to the limitations of supply, the dosage and the duration of treatment may have been inadequate.

Vinyl testosterone (Table VIII) was given to 12 women in doses varying from 300 to 500 mg. daily, usually for one- to two-week periods. In about half the patients there was a slight improvement, but only in one to a striking degree. Two patients showed untoward effects; one with ankle edema, another with hypomenorrhea. Again, the brief period of medication may have been a factor in the lack of success.

Our most extensive study was made with oral pregnenolone acetate

TABLE VI

THERAPEUTIC EFFECTS OF MARISONE (AYERST) IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	1200	1	4	4	
		2000	1	4	4	
2	F	1000	2	4	3	
3	F	1000	3	4	3	
4	F	1000	3	4	4	
		1000	2	4	4	More pain
5	F	1000	4	4	3	
6	F	1000	4	4	4	
7	M	1000	6	3–4	3	Felt better first 3 wks.
8	F	1000	6	4	4	
9	F	1000	6	3	3	Less pain—more energy
		2000	4	3	3	Nauseated—less pain
		1000	2	3	3	Vaginal bleeding
10	F	1000	7	3	3	
11	M	1000	8	3	3	Less pain
		800	6	3	3	Less pain
		1000	3	4	3	Nauseated
12	F	1000	8	3	3	Less pain
		1000	2	3	3	Less pain
		2000	3	4	3	Skipped period
13	F	1200	6	3	3	Less pain—nauseated and dizzy
14	F	2000	1	4	4	
15	M	2000	2	4	4	
16	F	2000	2	4	..	Constantly nauseated
17	F	2000	2	4	3	
18	F	2000	2	4	..	
19	F	2000	2	3	..	Slightly less pain and stiffness
20	M	2000	2	4	3	
21	F	2000	4	3–4	2	Slightly less pain first 2 wks.
22	F	2000	4	4	3	
23	F	2000	4	4	3	Vaginal bleeding
24	M	2000	4	4	3	
25	F	2000	4	4	..	Felt worse
26	F	2000	4	4	..	Vaginal bleeding
27	M	2000	4	4	4	
28	M	2000	6	4	4	
29	M	2000	6	4	..	Slightly less pain first 2 wks.
30	F	2000	6	3	3	

TABLE VII

THERAPEUTIC EFFECTS OF PREGNENETRIOLONE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	300	1	3	3	
2	F	300	1	4	2	Felt worse
3	F	300	1	4	2	Felt worse
4	F	300	1	4	4	
5	F	300	1	4	3	
6	F	300	2	4	2	
7	F	500	1	4	4	
8	M	500	2	3	3	Less pain
9	M	500	4	3	3	Less pain, more motion

TABLE VIII

THERAPEUTIC EFFECTS OF VINYL TESTOSTERONE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone*	*Remarks*
1	F	300	1	4	2	Edema of ankles
2	F	300	1	3–4	3	Slightly more motion
3	F	300	1	4	4	
4	F	300	1	3	3	Less pain
		500	2	3	3	Less pain
5	F	300	2	4	4	
6	F	400	2	4	4	
7	F	500	1	3	3	
		500	1	3	3	
8	F	500	1	4	3	
9	F	500	1	3	3	Less pain
		500	1	3	3	Less pain—diminished flow on period
		500	4	3	3	
10	F	500	2	4	4	
11	F	600	1	3	2	Slightly less pain
12	F	500	3	2	2	Marked improvement—skipped period

on which we have made previous preliminary reports.[1, 2] In the present study, 100 patients have been treated. These were 30 men and 70 women, ranging in age from 24 to 82, the mean for the group being 48.4 years.

The duration of illness varied from 6 months to 40 years, the mean period being 10.1 years. Of the entire group, 33 had a mild degree of anatomic progression of the disease (stage 2), 32 a moderate degree (stage 3), and 35 a marked degree of damage (stage 4). In regard to disability, 45 had a minor disability (class 2), 39 a moderate disability (class 3), and 16 were totally incapacitated (class 4). As a whole then the group consisted predominantly of older people with a chronic, disabling illness. There were no acute cases in the series. Before being referred to the clinic, the patients had usually had some other form of treatment over the years, resulting in little relief.

As a routine during the first two weeks, the patients were placed on placebo medication. If some improvement was noted, the placebo was repeated. If no improvement was observed, pregnenolone was administered in a dosage of 500 mg. daily. In a proportion of cases, relief of pain and greater mobility were noted in about two weeks. With continuation

TABLE IX

PERCENTAGES OF IMPROVEMENT, AGES, AND DURATION OF ILLNESS IN 100 PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH PREGNENOLONE

Grade of Improvement		*Age (Yrs.)*		*Duration of Disease (Yrs.)*	
	No.	*Mean*	*Range*	*Mean*	*Range*
Marked	30	44.5	28–71	8.9	0.5–29
Moderate	46	49.5	24–74	9.7	1.0–26
None	24	51.4	25–82	12.5	1.0–40
Total group	100	48.4	24–82	10.1	0.5–40

of pregnenolone medication, swellings subsided slowly in some instances. Where improvement was marked, the patients in most instances, except for a certain group, were taken off medication, usually with the substitution of placebos to determine the length of time before symptoms recurred. If no improvement occurred within a month, pregnenolone was usually withdrawn and another steroid substituted.

The results are shown in Table IX. Thirty patients showed a major degree of improvement, 46 a minor degree, and 24 patients no change. There was no sex differentiation in the effects; the proportion of men to women in each group was the same, about 1:2. In those patients who showed a striking change, there could be no question of the improvement. There was a marked diminution in pain, an increase in joint mobility, and an increase in energy and in the ability to accomplish tasks which had not been possible often for some years. In those patients who showed only mild or moderate degrees of improvement, the efficacy of the medication is open to question. Since the administration of placebos produces a mild improvement in about half the cases, as is shown in the testolactone and Δ^4-androstenedione studies, it is impossible to differ-

entiate the improvement incident to the psychotherapeutic effect of the treatment from that of the steroid itself. Caution is therefore essential in the evaluation of such cases.

The percentage of patients showing marked improvement has decreased from those obtained in our previous studies from 50 to 38 to 30. This is to some extent, we think, due to the increasing age and duration of illness of our clinic population. In one previous study, the average age and duration of illness in the nonimproved group were 43.7 and 9.5 years respectively. In the present study, these values have increased to 51.4 and 12.5 years. As can be noted in Table IX, the mean age and mean duration of illness increase steadily from those showing the greatest to those exhibiting the least degree of improvement.

In an attempt to evaluate such factors, we have analyzed the data for the relationship of the degree of improvement to chronological age, duration of illness, degree of anatomic progression, and degree of functional disability.

FIG. 1. The therapeutic response to pregnenolone in relationship to age in 100 patients with rheumatoid arthritis.

Fig. 1 shows the effect of age upon the level of improvement achieved. The patients are divided into three classifications of age ranges, each of which shows the percentage of major, minor, and no improvement in that group. It is evident that there is a decreasing tendency for major improvement to occur with increasing age, and an increasing tendency for no improvement to occur under similar conditions.

A similar analysis for the relationship of the duration of illness to degree of improvement is shown in Fig. 2. Since the mean duration of illness for the entire group is 10.1 years, the division has been made at that point. The more acute group shows a higher percentage of major improvement and a lower percentage of lack of improvement than the more chronic group.

In Fig. 3 is shown the relationship between the degree of anatomic progression of the disease and degree of improvement. It is evident that

as the disease becomes more marked, the percentage of striking improvement decreases and the percentage of unfavorable responses increases.

Finally in Fig. 4 the relationship of the amount of functional disability is correlated with improvement. Here again, patients with the least degree of limitation of function show the highest degree of improvement, while the incapacitated patients show the least therapeutic effect.

FIG. 2. The therapeutic response to pregnenolone in relationship to duration of illness in 100 patients with rheumatoid arthritis.

FIG. 3. The therapeutic response to pregnenolone in relationship to the degree of anatomical progression in 100 patients with rheumatoid arthritis.

From these data it is evident that the best results are obtained in the youngest, more acutely ill patients with the least amount of pathologic change. Furthermore, examination of the data reveals that 50 per cent of the patients in stages 1 and 2 in the age group from 20 to 39 show a major degree of improvement. Similarly, 50 per cent of the patients in the same age group placed in class 2 show major improvement. The degree of major improvement declines in the stage 1 to 2 groups with

increasing age to a low of approximately 17 per cent in the 60- to 82-year-old group. Similar declines in percentage of improvement occur with age in those placed in class 2. Major improvement was noted in 43.8 per cent of the 20- to 39-year-old group in stages 3 and 4 and 40 per cent of patients placed in classes 3 and 4 in the same age group. Again, stage 3 and 4 and class 3 and 4 patients show declining percentages of major improvement with increasing age. It is obvious, therefore, that oral pregnenolone should be productive of major improvement in approximately half of the arthritic patients, less than 40 years of age with mild involvement. Further analysis indicates that the relationship of age to degree of improvement is significant only in stages 1, 2, and 3 and classes 2 and 3. In those patients with marked anatomic progression (stage 4)

FIG. 4. The therapeutic response to pregnenolone in relationship to the degree of functional disability in 100 patients with rheumatoid arthritis.

and complete disability (class 4), there was no correlation with age. In short, marked disability minimizes the chances of improvement irrespective of age.

Brief mention may be made of the laboratory data for the three groups. The sedimentation rates tended to diminish and in some cases to reach normal levels in the major improvement group. The values during improvement were 74 per cent of the decreases, the mean level being 72 per cent of the control values. In the minor improvement group there was a lesser tendency for the sedimentation rates to fall, the mean value during treatment being 84 per cent of the control level. The eosinophil level was unaffected, at 98 per cent of the original value. In the group with no improvement, the mean sedimentation level after medication was 92 per cent of the original level, essentially unaffected.

Another factor that might have accounted for the failure of improvement in some patients was the inadequacy of the usual dosage of 500 mg. daily. Accordingly, in 24 patients the dose was increased to 1000 mg. daily (Table X). Four of these patients had responded well to

TABLE X

THE THERAPEUTIC RESPONSE TO LARGE (1000 MG.) DAILY DOSES OF PREGNENOLONE COMPARED WITH THAT OF AVERAGE (500 MG.) DAILY DOSES

Patient	*Sex*	*Daily Dose, Mg.*	*Duration of Treatment, Wks.*	*Grade*	*Grade with Pregnenolone, 500 Mg. Daily*	*Remarks*
1	F	1000	4/7	4	3	Felt worse and stopped med. after 4 days
2	M	1000	1	4	3	Felt worse and stopped med. after 1 week
3	F	1000	1 5/7	4	3	Nauseated after 12 days and stopped med.
4	F	1000	2	2	2	No improvement over smaller dose
5	M	1000	2	2	2	No improvement over smaller dose
6	F	1000	2	3	3	Slightly less pain
7	F	1000	2	3–4	4	
8	F	1000	2	4	4	
9	M	1000	2	4	4	
10	F	1000	2	4	3	Felt worse
11	M	1000	3	3	3	Had G.I. upset after 3 wks. and decr. dose to 600 mg.
12	F	1000	4	2	2	No improvement on bigger dose
13	F	1000	4	2	2	Felt better on larger dose
14	F	1000	4	2–3	3	Felt better on larger dose
15	F	1000	4	3	..	
16	F	1000	4	3	3	No improvement over smaller dose
17	M	1000	4	3	4	Less pain
18	M	1000	4	3	3	Slightly better than on smaller dose
19	F	1000	4	4	4	A little more energy
20	F	1000	4	4	3	Worse on larger dose
21	M	1000	4	4	4	
22	F	1000	4	3	3	Slightly less pain
23	F	1000	6	2	3	More energy—less pain
24	M	1000	6	3	3	No improvement over smaller dose

the previous dose; 13 had shown a mild effect; 6 had had no improvement; one had not received pregnenolone previously. The usual duration of time for the medication was two to four weeks. Three patients felt worse on the larger dose and discontinued the medication in less than two weeks. Three other patients persisted for longer periods of time but

also had an apparent exacerbation of symptoms. Five other patients gave no indication of any change on the larger dose. The other half of the patients noted mild improvement over their previous state with the increased amount of medication, but in only one (no. 23) was this at all striking. In spite, therefore, of this increased subjective response, an increase in grade of response did not seem evident. In general it may be concluded that the 500 mg. daily dose was optimal and that increases above this amount did not usually produce greater therapeutic benefits.

In view of the brief duration of the period of remission resulting after the discontinuance of ACTH and cortisone, it is of interest to ascertain how long the ameliorative effects of pregnenolone persist. To determine this, we have analyzed the data in 21 patients in whom there has been striking improvement, who showed a recurrence of symptoms after the drug was omitted, and who improved again after the resumption of the medication. In 13 of the patients, placebos were administered continuously so that they were unaware of any change in the procedure. Reinstitution of the medication was undertaken when the symptoms of relapse were definite. There was a good deal of variability in the length of time during which the patients remained in an improved state without pregnenolone and for this reason we have constructed a scatter diagram to determine whether the duration of the previous medication had any influence upon the length of the remission. This relationship is shown in Fig. 5. The open circles indicate the patients who received placebos between the intervals of pregnenolone medication, while the solid circles indicate those patients who had no medication during this period. In the majority of instances there seems to be a positive relationship between the length of time the patient receives pregnenolone and the length of time the improvement lasts without pregnenolone. There are some individual variations, some patients relapsing relatively rapidly and others more slowly. For most of the group, however, the relationship seems practically a 1:1 relation. That this relationship is not based on psychologic factors is shown by the fact that the distribution is the same whether the patient received placebos or no medication. This finding would seem to indicate that pregnenolone therapy maintained over a long period of time will lead to a prolonged period of improvement subsequent to termination of therapy.

In order to determine the course of rheumatoid arthritis under long-term pregnenolone therapy, we selected for study eight patients who responded favorably initially, but who relapsed under placebo medication and improved promptly on resumption of pregnenolone administration. This study group included two men and six women ranging in age from 31 to 53, the mean being 41 years. They had mild to moderate degrees of progression and a similar degree of incapacity, with the exception of one woman whose joints were ankylosed and who could get around only on crutches. They had suffered from arthritis from 3 to 14 years, the average being 7.4. Seven patients were given pregnenolone for 12 months; the eighth patient received the steroid for 10 months.

The general course during the period of therapy was characterized by increased activity and mobility with marked diminution in pains and swellings. Variation in the level of dosage showed that the optimal dose was about 500 mg. daily. The course of events in five patients was fairly uneventful, there being a fairly smooth maintenance of the level of improvement. In three patients, however, there were occasional fluctuations with minor temporary relapses unrelated to changes in dosage. There were no untoward effects whatsoever in any of the patients. The sedimentation rates fell, but remained abnormal in three. The circulating eosinophils varied widely initially from 50 to 881 per cubic millimeter,

FIG. 5. Relationship between duration of treatment with pregnenolone and the duration of remission without pregnenolone.

the average being 363. During the period of treatment, the values decreased in all but one case, the final average being 201. The blood cholesterol values taken at the end of the period of medication were within normal limits, all being below 200 with the exception of one value which was 260. Thus, pregnenolone did not produce the hypercholesterolemia observed after the administration of ACTH or cortisone.[4] In evaluating the results we feel that the medication did not achieve the levels possible with ACTH or cortisone but did succeed in maintaining these individuals at a relatively normal degree of comfort and activity with no accompanying ill effects.

The ameliorative effects of cortisone by mouth were studied in 12 subjects. These patients were hospitalized for three weeks so that various

biochemical analyses could be made upon them before and during the course of the treatment. The cortisone was administered routinely in a dosage of 300 mg. for the first 24 hours, 200 mg. daily for the next 48 hours, and 100 mg. daily thereafter. In the course of two weeks the cumulative dosage was 1.8 gm. They were then given (in nine cases) pregnenolone by mouth in a daily dosage of 500 mg. in an attempt to maintain the degree of improvement produced. The results of the biochemical studies will be discussed by Pincus.[3]

TABLE XI
THERAPEUTIC RESULTS WITH ORAL CORTISONE

Patient	*Sex*	*Age, Yrs.*	*Dur. of Dis., Yrs.*	*Stage*	*Class*	*Dose in 2 Weeks, Gm.*	*Grade*	*Follow-up*
1	F	42	1.5	1	3	1.8	2	Relapse in 10 days on pregnenolone
2	F	58	4	4	3	1.8	2	Relapse in 1 week on pregnenolone
3	F	75	9	4	4	1.8	3	Maintained on pregnenolone for 1 month
4	F	40	8	3	2	1.8	2	Maintained on pregnenolone for 2 weeks
5	F	41	1.5	4	4	1.8	3	Maintained on pregnenolone for 2 months
6	F	61	3	4	3	1.8	2	Relapse in 1 week on pregnenolone
7	M	62	16	3	3	1.8	2	No pregnenolone. Relapse in 1 week
8	M	43	13	4	4	1.8	3–4	Slightly more improvement on injected cortisone
9	M	42	5	3	3	1.8	2	Relapsed steadily on pregnenolone
10	M	51	6	3	3	1.8	2	Maintained on pregnenolone for 3 weeks
11	M	52	2	4	3	1.8	2	Maintained on pregnenolone for 10 days
12	M	62	10	4	3	1.8	3	No pregnenolone. Relapsed in 2 days

The results of the treatment are shown in Table XI. In eight patients there was striking improvement which began within 72 hours, as evidenced by diminution of pain, increased mobility, and decrease in swellings. In three patients there was only mild to moderate improvement which consisted entirely in relief of pain. These (nos. 3, 5, 12) were individuals with marked destructive changes in their joints. In one case (no. 8) there was hardly any relief; this individual was an extremely

debilitated, bedridden patient who experienced slightly more improvement when cortisone was administered subsequently by injection. It is of interest that the sedimentation rates during the period of medication were essentially unaffected. In no case was there a decrease to a normal level.

As these patients have been studied recently in sequence, there has been a variable period in which to observe their subsequent course. Two of the patients had no further medication and relapsed promptly, one (no. 12) within two days and the other (no. 7) within a week. Nine patients have been given pregnenolone subsequent to the discontinuance of the cortisone. Five relapsed within a week to ten days. Four have maintained their improvement over periods ranging from two weeks to two months, insofar as our observations to date are concerned. While the data are obviously inadequate to draw any conclusions, there is a suggestion that pregnenolone may be of value in maintaining the effects of cortisone for a variable length of time.

Summary

A variety of studies has been made in cases of rheumatoid arthritis with orally administered testolactone, androstenedione, pregnenetriolone, vinyl testosterone, 16-dehydropregnenolone, dehydroisoandrosterone, and Marisone. None of these steroids has shown any definite ameliorative effects. Further studies in 100 patients with rheumatoid arthritis treated with oral pregnenolone showed striking improvement in 30, a mild improvement in 46, and no improvement in 24. Sedimentation rates tended to decrease in patients showing favorable clinical responses. Doubling the usual dose of 500 mg. daily produced no more definite therapeutic benefits. Pregnenolone was administered for a year to eight patients with satisfactory results and no untoward effects. Twelve patients treated with cortisone orally resulted in eight patients with striking improvement, three with mild improvement, and one with no improvement; but sedimentation rates did not decrease. Pregnenolone appeared to prolong the remission in four out of nine cortisone-treated patients.

The studies reported indicate the need for careful evaluation of the situational factors inherent in any therapeutic endeavor. The necessity for carefully controlled observations, even to ensure the impartiality of the observer, is indicated in the investigations with testolactone and androstenedione, since approximately half of a given number of patients will indicate some degree of improvement with placebo medication. Accordingly, mild responses to medication cannot be considered in evaluating the efficacy of any drug in this disease.

Pregnenolone is efficacious in a certain percentage of patients, but chiefly in the earlier stages of rheumatoid arthritis where the pathologic process has not advanced too far. The resulting improvement lasts much longer than that following cortisone or ACTH. Pregnenolone therapy can if necessary be maintained indefinitely without harmful effects.

Pregnenolone may be of value in some cases in prolonging the remission following the administration of cortisone.

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Discussion

Discussion on this paper follows Chapter 14.

14

Pregnenolone in the Treatment of Rheumatic Diseases with Particular Emphasis on Soft Tissue Involvement

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The wide divergence of reports on the treatment of rheumatoid arthritis with pregnenolone, varying from very good[1, 2, 3, 4] to very poor[5, 6, 7, 8, 9, 10] benefit, prompted a search for a common denominator to account for these results. A critical study of several groups of patients revealed some interesting facts.

In one group of 12 patients suffering from "rheumatoid arthritis" and benefited by pregnenolone, 3 patients had osteoarthritis, 5 fibrositis and 4 had rheumatoid arthritis.

A second group of 15 patients considered unimproved by pregnenolone were found to have active rheumatoid arthritis in every case.

From these observations and from personal experience it appeared that when diagnostic criteria were lax and the patient had more comfortable function the result was considered good. On the other hand, when a critical evaluation of the effect on the rheumatoid activity was the basis for improvement, none was seen.

Evaluation of the types of improvement seen after the treatment of various rheumatic diseases with pregnenolone revealed these to consist of decreased fatigue, relief of stiffness after inactivity, decreased pain, improved function, and decreased swelling. These beneficial effects usually appeared in this order. With the exception of the swelling, the improved features were those caused by fibrositis. In each instance where the patient was improved, whether the rheumatic condition was rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, bursitis or fibrositis, the predominant symptoms complained of were those of fibrositis. Thus, improvement in these symptoms made the patient more comfortable and thereby increased the function. These findings led to a study of pregnenolone in the treatment of fibrositis.

Subjects and Methods

The patients selected for this study were only those who had no evidence of active rheumatoid arthritis, rheumatoid spondylitis or symptomatic osteoarthritis. To evaluate the material the patients were classified as to severity of aching and stiffness (1 to 4, symptoms relieved by 5–10 minutes of activity to constant discomfort in spite of activity), and the response to therapy (1 to 4, complete recovery to no improvement).

The series consisted of 60 patients (Table I), 46 men and 14 women with an age range of 28 to 69 years, averaging 46 years. The duration of symptoms was from 4 days to 6 years, with an average of 11 months. Nineteen patients had mild to moderate disability while 41 had moderately severe to severe difficulty. The limitation of function roughly paralleled the severity of the disease.

TABLE I

AREA OF INVOLVEMENT

Subdeltoid Bursitis	10
Fibrositis	
Cervical	15
Lumbosacral	15
Elbow	5
Hands	10
Knees	5
Total	60

If the history suggested any possible arthritic difficulty, roentgenograms, sedimentation rates and blood cell counts were done to determine whether active rheumatoid arthritis, rheumatoid spondylitis or osteoarthritis were present. If the studies indicated a possible arthritic basis the patients were not included in the series.

For the treatment of subdeltoid bursitis, which was acute in each case, the longest duration being 5 days, 400 mg. of pregnenolone were given intramuscularly, in a single daily dose. The duration of treatment was 6 days to 14 days followed by 800 mg. of oral medication for 8 to 10 days. In treating the various forms of fibrositis 600 mg. to 1 gm. were given orally for an average duration of 8 weeks. The shortest period was 3 weeks and the longest 12 weeks. Each patient was given 600 mg. for 2 weeks; if satisfactory improvement had not occurred within 2 weeks the dose was increased to 800 mg. for the next 2 weeks and if lack of improvement continued the amount of the drug was increased to 1 gm. daily. Pregnenolone acetate in 100 mg. tablets was used.

Results

Acute Bursitis. Moderate to marked improvement (Table II) occurred in 7 of 10 patients (4 men, 3 women) with acute bursitis of 3 to

5 days duration. There was relief of pain, muscle spasm and limited motion within 3 to 6 days after treatment was started. One man and one woman were more comfortable on the second day of treatment. In all instances only slight tenderness remained by the fourteenth day of treatment although slight muscle guarding was apparent in three patients at

TABLE II

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF SUBDELTOID BURSITIS

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Moderate	2	.	.	.
Moderately Severe	1	1	.	1
Severe	2	1	1	1

the twenty-fourth day. Three patients with marked improvement received a total of 6 days intramuscular pregnenolone and 8 days of oral pregnenolone acetate. One patient had mild improvement over 24 days of treatment, exhibiting only relief of pain while continuing moderate muscle spasm and limitation of motion. The two failures presented moderately severe tenderness, splinting of the arm and very limited motion after 24 days of treatment.

A control series of 4 patients was treated with daily injections of a cholesterol suspension for 24 days with no improvement over that period of time.

Fibrositis. Marked improvement (Table III), was noted in 9 pa-

TABLE III

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS IN VARIOUS AREAS

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	6	2	.	.
Moderate	1	4	4	.
Moderately Severe	2	6	5	1
Severe	.	8	7	4

tients (7 men, 2 women). In these instances there was complete remission of aching, stiffness, limitation of motion and fatigue. No improvement was noted during the first 7 days but all patients began to respond within the second week, characteristically exhibiting decreased fatigue with progressive improvement in stiffness, aching and motion. The shortest period of treatment was 5 weeks, the longest 12 weeks with an average of 8.5

weeks. No relapses have occurred over a period of 3 to 5 months since pregnenolone was discontinued.

A moderate response to therapy occurred in 20 patients (15 men, 5 women) over a period of 12 weeks. These patients continued to have very transient stiffness after inactivity of several hours, which was relieved within ten to twenty seconds of normal activity. Fatigue was not abnormal. Again improvement was noted in 13 patients in the second week, in 5 patients in the fourth week and in 2 patients in the seventh week. Relapses occurred in 6 patients (3 men, 3 women) within 4 to 7 weeks after therapy was discontinued. Reinstituting therapy of 800 mg. daily again relieved the symptoms to a tolerable degree within 2 to 3 weeks in each instance. All patients have been observed for 3 to 5 months since therapy was discontinued.

Only mild improvement, consisting of reduced fatigue, aching and stiffness with increased motion, was achieved in 16 patients (13 men, 3 women). Satisfactory improvement in these patients was not noted before the sixth week of treatment which means each of these patients

TABLE IV

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS IN CERVICAL REGION

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	1	1	.	.
Moderate	.	2	.	.
Moderately Severe	.	3	3	1
Severe	.	2	1	1

received 1 gm. of pregnenolone beginning with the fifth week of treatment. Relapses occurred in 11 patients (8 men, 3 women) in this group in 3 to 7 weeks after discontinuance of therapy. Within 3 to 4 weeks after therapy was again instituted with 1 gm. of pregnenolone a satisfactory response was achieved. No relapses have occurred in the 8 to 9 weeks since the second course of therapy was given.

Of the 50 patients with fibrositis in various areas only 5 patients (4 men, 1 woman) failed to make any improvement with as much as 1 gm. of pregnenolone daily. No reason for this failure could be found with the possible exception that all of these patients were hypersensitive to pain and extremely apprehensive of possible crippling effect of their ailment.

There were no toxic effects with pregnenolone or pregnenolone acetate. Even daily doses of 1 gm. were well tolerated.

An examination of Tables II and III indicates that the 7 failures occurred in the more serious cases (5 severe, 2 moderately severe). As the severity decreases the moderate to marked improvement increases.

Fibrositis of the cervical area was completely relieved in only one

mild case, Table IV. Significant improvement was evenly divided through the various degrees of severity with slight benefit in 4 of the more severe cases. There were 2 therapeutic failures.

Significant improvement was evident in 7 patients (6 moderate, 1 marked), Table V, in severe fibrositis of the lumbosacral region. The only

TABLE V

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS OF LUMBOSACRAL REGION

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	1	.	.	.
Moderate	.	1	1	.
Moderately Severe	.	2	.	.
Severe	.	3	4	3

TABLE VI

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS OF ELBOWS

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	1	.	.	.
Moderate	.	1	1	.
Moderately Severe	.	1	.	.
Severe	.	1	.	.

TABLE VII

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS OF HANDS

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	2	1	.	.
Moderate	1	.	1	.
Moderately Severe	2	.	1	.
Severe	.	1	1	.

marked improvement again occurred in a mild case. In this group failure or mild benefit outnumbered the significant improvement 8 to 7.

Fibrositis of the elbows responded moderately in 3 of 5 patients, Table VI, with one complete relief of a mild case and one mild result of a moderate involvement.

Complete relief of symptoms occurred in one-half (5 patients) of the

fibrositis of the hands, Table VII. In addition, moderate relief occurred in 2 patients and mild benefit in 3.

The benefit achieved in fibrositis of the knees, Table VIII, was not outstanding. There was significant improvement in 2 cases (1 marked, 1 moderate) while 3 were only slightly improved.

From this data it would appear that the greatest benefit was obtained in subdeltoid bursitis and fibrositis of the hands, followed by fibrositis of

TABLE VIII

RESPONSE TO PREGNENOLONE IN RELATION TO SEVERITY OF FIBROSITIS OF KNEES

	Response to Therapy			
Severity	*1 Marked*	*2 Moderate*	*3 Mild*	*4 None*
Mild	1	.	.	.
Moderate	.	.	1	.
Moderately Severe	.	1	1	.
Severe	.	.	1	.

the elbows, cervical region, lumbosacral area and knees in descending order of improvement.

Comment

The results of this study indicate that 58% (29 patients) of these patients made a moderate to marked improvement under this plan of study. Another 32% (16 patients) showed a mild improvement. Only 10% (5 patients) failed to improve at all. Thus 90% (45 patients) derived some benefit from pregnenolone therapy.

When this group of patients is compared with similar groups treated with salicylates or a rehabilitation program we find essentially the same results although the therapeutic response does not occur as rapidly and the duration of treatment may be extended by many months. What is the specific effect of pregnenolone in these rheumatic diseases? Does it have an analgesic effect? Does it affect the soft tissues directly or is there an effect through the endocrine system? These questions may appear unanswerable at first glance but possibly there is a very simple explanation for most of them.

In the first place, fibrositis is not a mysterious disease but the result of a very simple common occurrence. It is the natural symptomatic response of muscular atrophy brought on by disuse, decreased use or strain. It may occur after a few weeks of disuse or after months to years of decreased use or strain. In other words, muscle which has never been trained to any peak of efficiency needs very little activity to maintain its tone, but when a muscle has reached a peak of efficiency it must be maintained at that level or tapered downward gradually. Any sudden drop or too rapid tapering will bring on the symptoms of fibrositis.

These are provable facts. Without the aid of any medication, grad-

uated exercises, on an ascending scale always below the tolerance level for fatigue will completely alleviate the symptoms of fibrositis. When the muscle rehabilitation has reached the point where it erases the fibrositic symptoms, the level can be maintained by simple daily physical activity of a lesser degree than the rehabilitation program.

Salicylates, heat and mild activity which give symptomatic relief to the fibrositis patient ease the symptoms but fail to raise the tolerance level for fatigue. This is probably the crux of the entire problem. Muscle exercise up to the tolerance level is maintenance or rehabilitative activity, but past the point of fatigue is productive of muscle strain and atrophy. This prolongs the illness and the convalescence.

Pregnenolone not only combats fatigue[11] but raises the tolerance level for fatigue. Under the influence of this drug a patient is not only capable of greater activity but actually indulges in more activity since fatigue is less limiting. Therefore, pregnenolone probably has an endocrine effect which raises the individual tolerance level for fatigue, permitting greater physical activity than before, which rehabilitates the muscle more rapidly than can be anticipated with simple symptomatic relief.

In bursitis, the muscle spasm caused by voluntary and involuntary splinting of the shoulder is eased, decreasing the pressure and irritation of the bursa by the spastic muscle which permits the local inflammation to subside more rapidly.

In rheumatoid arthritis, pregnenolone has no specific action upon the rheumatoid activity. It again, benefits the fibrositis which is a secondary feature of muscle atrophy from disuse and strain in protecting the painful joint. By elevating the tolerance level for fatigue, the muscle begins to regain its former development; this permits better function and promotes circulation in the part which may to a certain extent reduce the swelling. Pregnenolone can do this only when the fibrositis predominates. When the rheumatoid activity is most prominent, it is valueless.

Summary

Pregnenolone in doses of 400 mg. to 1 gm. daily for 2 to 12 weeks, was used in the treatment of 10 patients with acute subdeltoid bursitis and 50 patients with simple uncomplicated fibrositis. Nine patients had a marked response; 20 were moderately improved; 16 were mildly benefited, and 5 showed no improvement. No toxic effects occurred.

From this study it appears that the greatest benefit from pregnenolone therapy may be subdeltoid bursitis followed by fibrositis of the hands, elbows, cervical region, lumbosacral area and knees.

The action of pregnenolone in these rheumatic conditions is probably through the endocrine system by elevating the tolerance level for fatigue, permitting more rapid muscle rehabilitation which alleviates the symptoms and restores normal function.

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The pregnenolone and pregnenolone acetate for this study were supplied by Sharp & Dohme, Chemical Specialties Co., Inc., and Schering Corporation.

Discussion of Chapters 11, 12, 13, and 14

W. Bauer: Drs. William S. Clark, Henrik O. Tonning, and I have made similar observations on patients with rheumatoid arthritis before, during, and after the administration of 11-desoxy steroids. All 11-desoxy steroids used in our study, with the exception of the pregnenolone (intramuscular), were made available to us through the courtesy of Dr. I. V. Sollins, Chemical Specialties Co., Inc., New York, New York. Our experience to date has been limited to four groups of cases, a total of 27

patients who received such compounds. The pertinent data pertaining to these four groups of patients will be presented in tabular form. It will be noted that our results are quite different from those reported by the four previous speakers.

The pre- and post-treatment status of the rheumatoid arthritis (state of the disease, functional impairment, and response to therapy) of each patient where judged according to the criteria adopted by the American Rheumatism Association. Only one patient had stage 1 rheumatoid arthritis. In the other 26 patients the disease was classified as stage 2 or stage 3. In our opinion, patients with stage 1 rheumatoid arthritis of less than one year's duration should be excluded from any study aiming to evaluate the therapeutic efficacy of a given antirheumatic agent because the natural remission rate is very high in such cases. This statement is based on a follow-up study of 250 patients treated with simple medical and orthopedic measures [Short, C. L., and Bauer, W., New Eng. J. Med., 238, 142, (1948)]. Of the 81 patients in this group with rheumatoid arthritis of one year's duration or less, approximately 75 per cent showed some degree of improvement and 37 per cent were in remission. The percentage of improvement in patients seen within the first six months of their disease was even higher, 81 per cent. As might be expected, improvement was less frequent and remission less complete in patients with rheumatoid arthritis of years' duration. Although frequently neglected, these findings should always be taken into consideration in the evaluation of methods of therapy.

That stage 1, stage 2, and stage 3 rheumatoid arthritis of years' duration is affected favorably by agents possessing antirheumatic activity has been demonstrated repeatedly. In cortisone- and ACTH-induced remissions the improvement in symptoms and signs is equally as prompt as in remissions associated with jaundice and pregnancy. The rapidity and the degree of improvement noted in hormone-induced remissions are directly related to the dose employed. Consideration of these and other factors is important when assaying agents for antirheumatic activity.

In a chronic disease of unknown cause, such as rheumatoid arthritis, characterized by unpredictable spontaneous remissions and exacerbations, it is extremely difficult to evaluate the results of treatment with any degree of certainty unless the studies are rigidly controlled. Because of the decided variations in the course of the disease from day to day, each patient must be made to serve as his own control. If studies pertaining to the evaluation of therapeutic procedures are not conducted in this manner, one is likely to conclude that the improvement observed is the result of treatment, whereas it may actually represent a natural variation in the course of the disease. Such a controlled study can be made only by choosing patients whose clinical course and variation in sedimentation rate have been known for months or years prior to the institution of a new form of treatment. An adequate follow-up period is most essential, so that one can decide as to the permanency of any improvement noted during the period of treatment. In addition to these precautions, it is extremely

important that the patient be kept on the same basic regimen before, during, and after the administration of the treatment being evaluated.

Every available means should be employed in evaluating clinical improvement. One should record separately the subjective, objective, and laboratory evidence of improvement, the final results being based on all three. This method of recording enables one to establish more accurately what, if any, psychic effect must be taken into account in the final summation. In all fairness to the type of treatment being studied, only patients whose disease is active and still reversible should be selected. If the administration of a given form of treatment should result regularly in improvement in patients with rheumatoid arthritis, one then has reason to suspect that one is dealing with an agent possessing antirheumatic activity.

The data pertaining to the 13 patients who received pregnenolone orally, 800 mg. per day, are presented in Table I. Placebo treatment was administered to all patients for at least two weeks: five received it prior to pregnenolone therapy, three after, and five before and after. It will be noted that the slight subjective improvement observed in the first five patients during placebo therapy was not enhanced when pregnenolone was administered. In only four patients was slight subjective improvement observed with the institution of pregnenolone treatment. In only one patient did we observe slight objective improvement. In this instance the improvement in the knee may have been due to a brace which was acquired during the period of treatment.

Prior to the administration of pregnenolone these 13 patients had been on a conservative regimen. The only medication employed was aspirin. It was administered to 12 of the 13 patients. It is of interest that seven of the patients found it necessary to continue their daily dose of aspirin during pregnenolone therapy because of the severity of pain. Two patients discontinued aspirin, only to resume it because of pain. Only three patients were willing to go through the entire period of pregnenolone treatment without a daily ration of aspirin.

The effect of pregnenolone on the sedimentation rate is depicted in Fig. 1. It will be noted that pregnenolone therapy had no significant effect on the sedimentation rate, whether judged on the basis of individual cases, on the mean sedimentation rate curve for the entire group, or on the calculation of mean differences. As will be seen in the same figure, these findings are in marked contrast to those observed in one patient during a typical cortisone response and another patient during a typical ACTH response.

In order to evaluate further the antirheumatic activity of pregnenolone, we studied two patients (whose cases were not included in Tables I to IV) in the manner indicated in Fig. 2. In these experiments the patients were informed that two steroids, both white emulsions, were to be given intramuscularly in order to determine whether they possessed antirheumatic activity. However, the nature of the steroids employed and when they were administered remained unknown to the patients:

TABLE I

RESULTS OF ORAL PREGNENOLONE THERAPY IN RHEUMATOID ARTHRITIS

Number of patients treated:	7 female, 6 male	13 patients	
Age:	35 to 70 years	47.8 years, average	
Duration of disease:	3.5 to 25 years	13 years, average	
Duration of exacerbation:	6 to 48 months	18 months, average	
Severity:	Moderate	11 patients	
	Mild	2 patients	
A.R.A. classification:		*Grade II*	*Grade III*
Stage of disease		7 patients	6 patients
Functional impairment		4 patients	9 patients
Method of study:			
Control period on placebo prior to pregnenolone		5 patients	
Control period on placebo after pregnenolone		3 patients	
Control period on placebo before and after pregnenolone		5 patients	
Dose:		800 mg./day	
Duration of therapy:		42 days—11 patients	
		56 days— 1 patient	
		30 days— 1 patient	
Side-effects:		None	
Results:			
Slight subjective improvement			4 patients
On placebo only		1 patient	
Beginning on placebo and continuing unchanged on pregnenolone		4 patients	
Beginning on placebo but further improvement on pregnenolone		1 patient	
On pregnenolone only		1 patient*	
Beginning on pregnenolone and maintained on placebo		2 patients	
Unchanged or worse		4 patients	
Slight objective improvement (acquired brace during therapy)			1 patient*
Therapeutic results judged according to A.R.A.:		Grade IV—13 patients	
		Grade III— 0 patients	
		Grade II— 0 patients	
		Grade I— 0 patients	
Additional therapy:			
Aspirin:			
Continued throughout because of severity of pain		7 patients†	
Discontinued but resumed because of pain		2 patients‡	
Discontinued throughout course		3 patients	
No aspirin prior to pregnenolone		1 patient	
Medication prior to study: Aspirin		12 patients	
Sedimentation rate: No significant change		13 patients	

* Same patient.

† In one patient the total daily dose of aspirin was reduced from 40 to 50 grains to 20 to 30 grains.

‡ One patient returned to the original dose of aspirin; the other required only 30 grains during the last two weeks of pregnenolone therapy.

As can be seen in Fig. 2, the daily administration of 300 mg. of pregnenolone to Mrs. T. C. for a two-week period was without effect on the rheumatoid arthritis. With the institution of cortisone therapy in doses of 300, 200, and then 100 mg. per day, a good antirheumatic effect, accompanied by a fall in the sedimentation rate to normal, was observed. After 14 days of cortisone treatment, pregnenolone was readministered, unbeknown to the patient, in doses of 900 mg. per day for two weeks. On the

FIG. 1. Rheumatoid Arthritis—Sedimentation Rate (Rourke-Ernstene).

tenth day of pregnenolone therapy, the previously noted antirheumatic effect began to disappear. By the fourteenth day the arthritis was essentially the same as during the precortisone period and the sedimentation rate was again elevated. Reinstitution of cortisone therapy in the same dosage previously given again had a good antirheumatic effect. The other patient so treated responded in much the same manner, experiencing no antirheumatic effect with pregnenolone and a good antirheumatic response with cortisone. In neither instance was it possible to maintain the beneficial effect of cortisone by the subsequent administration of large doses of pregnenolone. In the second case the arthritis returned to its precortisone status 48 hours after the institution of pregnenolone therapy in doses of 900 mg. per day.

Five additional patients were treated with pregnenolone intramuscu-

TABLE II

RESULTS OF INTRAMUSCULAR PREGNENOLONE IN RHEUMATOID ARTHRITIS

Number of patients treated:	4 male, 1 female	5 patients	
Age:	21 to 56 years	45 years, average	
Duration of disease:	3 to 25 years	12.5 years, average	
Duration of exacerbation:	6 months to 11 years	3.1 years, average	
Severity:	Mild	1 patient	
	Moderate	3 patients	
	Severe	1 patient	
A.R.A. classification:	*Grade I*	*Grade II*	*Grade III*
Stage of disease	1 patient	1 patient	3 patients
Functional impairment	0 patients	1 patient	4 patients
Method of study:			
Control period before and after pregnenolone		5 patients	
Dose and duration of therapy:			
500 mg./day for 6 weeks (21 gm.)		2 patients	
500 mg./day for 4 weeks (14 gm.)		1 patient	
300 mg./day for 4 weeks (8.4 gm.)		1 patient	
300 mg./day for 2 weeks; 200 mg./day for 1 week (5.6 gm.)		1 patient	
Side-effects: Induration with questionable abscess		1 patient	
Results:			
Slight subjective improvement			4 patients
On pregnenolone alone		2 patients	
On pregnenolone and continued unchanged		2 patients*	
Unchanged or worse		1 patient	
Slight objective improvement			1 patient*
On pregnenolone and continued unchanged		1 patient	
Unchanged or worse		4 patients	
Therapeutic results judged according to A.R.A. criteria:		Grade IV—5 patients	
		Grade III—0 patients	
		Grade II—0 patients	
		Grade I—0 patients	
Additional therapy:			
Aspirin			
Continued throughout because of severity of pain		3 patients†	
Discontinued throughout course only		2 patients	
Medication prior to study:			
Aspirin		5 patients	
Sedimentation rate: No significant change		5 patients	

* Same patient.

† No change in total dose of aspirin required.

larly. All intramuscular pregnenolone used in the study was supplied through the courtesy of Dr. G. E. Farrar, Jr., of Wyeth, Incorporated, and Dr. M. L. Tainter, Director of Sterling-Winthrop Research Institute. The data pertaining to these patients are tabulated separately in Table II, because it is the only group of patients treated solely with parenteral pregnenolone. The subjective and objective improvement noted in this small group of patients, when judged according to American Rheumatism Association criteria, was very slight and therefore they are recorded as grade IV.

FIG. 2. T.C. Female, 59. Rheumatoid Arthritis.

16-Dehydro pregnenolone was administered to three patients in doses of 1 gm. per day. Two patients were treated for 14 days, one for 30 days. That this 11-desoxy steroid exhibited no antirheumatic effect in these three patients is clearly shown in Table III.

Four patients received Δ^5-pregnene-3β, 21-diol-20-one-21-propionate intramuscularly. The data pertaining to these patients and their therapeutic response are shown in Table IV. Only one patient experienced slight subjective and objective improvement. The final grading in all patients was grade IV.

Only two patients were treated with saturated desoxycorticosterone

TABLE III

RESULTS OF ORAL 16-DEHYDRO PREGNENOLONE IN RHEUMATOID ARTHRITIS

Number of patients treated:	2 females, 1 male	3 patients	
Age:	22 to 45 years	34 years, average	
Duration of disease:	3½ to 8½ years	6 years, average	
Duration of exacerbation:	¾ to 3 years	1.9 years, average	
Severity:	Mild	1 patient	
	Moderate	1 patient	
	Severe	1 patient	
A.R.A. classification:		*Grade II*	*Grade III*
Stage of disease		2 patients	1 patient
Functional impairment		1 patient	2 patients
Method of study:			
Control period before and after drug		1 patient	
Control period before and currently on therapy		2 patients	
Dose:		1 gm./day	
Duration of therapy:		30 days—1 patient	
		14 days—2 patients	
Side-effects:		None	
Results:			
Subjective improvement			0 patients
Unchanged or worse		3 patients	
Objective improvement			0 patients
Unchanged or worse		3 patients	
Therapeutic results judged according to A.R.A. criteria:		Grade IV—3 patients	
		Grade III—0 patients	
		Grade II—0 patients	
		Grade I—0 patients	
Additional therapy:			
No aspirin during study		2 patients	
Aspirin resumed because of pain		1 patient	
Medication prior to study:			
Aspirin		2 patients	
No aspirin		1 patient	
Sedimentation rate: No significant change		3 patients	

intramuscularly. Again the improvement noted in the one patient was so slight that it was recorded as grade IV (see Table V).

Our experience with four 11-desoxy steroids, excluding those patients

TABLE IV

RESULTS OF INTRAMUSCULAR INJECTION OF Δ^5-PREGNENE-3β, 21-DIOL-20-ONE-21-PROPIONATE IN RHEUMATOID ARTHRITIS

Number of patients treated:	All males	4 patients	
Age:	34 to 66 years	50 years, average	
Duration of disease:	1 to 50 years	17 years, average	
Duration of exacerbation:	1 to 3 years	1¾ years, average	
Severity:	Moderate	2 patients	
	Mild	1 patient	
	Severe	1 patient	
A.R.A. classification:	*Grade II*	*Grade III*	*Grade IV*
Stage of disease	3 patients	1 patient	
Functional impairment		3 patients	1 patient
Method of study:			
Control period before and after drug		4 patients	
Dose:		300 mg./day	
Duration of therapy:		20 days—2 patients 15 days—2 patients	
Side-effects: Local induration		4 patients	
Results:			
Slight subjective improvement			1 patient*
Began on drug and maintained in control period		1 patient	
Unchanged or worse		3 patients	
Objective improvement			1 patient*
Slight on drug and maintained in control period		1 patient	
Unchanged or worse		3 patients	
Therapeutic results judged according to A.R.A. criteria:		Grade IV—4 patients Grade III—0 patients Grade II—0 patients Grade I—0 patients	
Additional therapy:			
Aspirin continued unchanged		2 patients	
No aspirin during study		2 patients	
Medication prior to study:			
Aspirin		4 patients	
Sedimentation rate: No significant change		4 patients	

* Same patient.

TABLE V

RESULTS OF INTRAMUSCULAR SATURATED DESOXYCORTICOSTERONE IN RHEUMATOID ARTHRITIS

Number of patients treated:	All males	2 patients	
Age:	31 to 53 years	42 years, average	
Duration of disease:	5 to 30 years	17½ years, average	
Duration of exacerbation:	1 to 2½ years	1¾ years, average	
Severity:	Moderate	2 patients	
	Mild	0 patients	
	Severe	0 patients	
A.R.A. classification:		*Grade II*	*Grade III*
Stage of disease		2 patients	
Functional impairment			2 patients
Method of study:			
Control period before and after drug		2 patients	
Dose:		100 mg./day	
Duration of therapy:		30 days—2 patients	
Side-effects: Local induration		1 patient	
Results:			
Slight subjective improvement			1 patient*
Began on drug and maintained in control period		1 patient	
Unchanged or worse		1 patient	
Objective improvement			1 patient*
Slight on drug and maintained in control period		1 patient	
Unchanged or worse		1 patient	
Therapeutic results judged according to A.R.A. criteria:		Grade IV—2 patients Grade III—0 patients Grade II—0 patients Grade I—0 patients	
Additional therapy:			
No aspirin during study		2 patients	
Medication prior to study:			
Aspirin		2 patients	
Sedimentation rate:			
No significant change		2 patients	

* Same patient.

who received both cortisone and pregnenolone, is summarized in Table VI. Here one notes that the therapeutic response in all 27 patients was classified as grade IV, unchanged or worse. This final classification of therapeutic response requires further comment in light of the fact that in Tables I to V, inclusive, one finds 10 patients listed as having experienced slight subjective improvement, of whom four exhibited slight objective improvement when steroid therapy was instituted.

As previously stated, the therapeutic response was judged according to the criteria recommended by the American Rheumatism Association. In the report of the Association, it was emphasized that "the criteria are

TABLE VI

Steroid	*Daily Dose (mg.)*	*Route of Administration*	*Number of Patients*	*Therapeutic Results, Judged According to A.R.A. Criteria*
Pregnenolone	800	Oral	13	Grade IV
Intramuscular pregnenolone	200 to 500	Intramuscular	5	Grade IV
16-Dehydro pregnenolone	1000	Oral	3	Grade IV
Δ^5-Pregnene-3β, 21-diol-20-one-21-propionate	300	Intramuscular	4	Grade IV
Saturated Desoxycorticosterone	100	Intramuscular	2	Grade IV

based entirely on *objective evidence* since subjective symptoms are considered unreliable." The objective improvement noted in the four patients was so slight and so short-lived as to be judged insufficient to fulfill the criteria required for classification as grade III (minor improvement). The objective improvement noted was no greater than had been observed in these same patients during like periods of time while receiving a simple medical regimen and aspirin. The continued elevation of the sedimentation rate in all cases during 11-desoxy steroid therapy supports our interpretation of the observed clinical response.

In screening drugs for antirheumatic activity, the criteria used for judging therapeutic response must be sufficiently reliable to detect agents of low potency. That the criteria employed in this study permit detection of antirheumatic activity comparable to that of aspirin was clearly demonstrated when evaluating the antirheumatic activity of pregnenolone. In only five of the 18 patients was it possible to discontinue the daily ration of aspirin during the period that this 11-desoxy steroid was administered.

On the basis of our experience to date we must conclude that no one

of the four 11-desoxy steroids, in the dosage used and the period of time administered, possessed demonstrable antirheumatic activity.

We are interested in the evaluation of 11-desoxy steroids because of the therapeutic implications suggested by the studies reported by Dr. Zaffaroni and the Worcester group. If further studies concerning the evaluation of the antirheumatic activity of 11-desoxy steroids are to be undertaken, the workers concerned should agree in advance on methods of procedure, including selection of patients and criteria for judging therapeutic response, as well as the recording and the analysis of data. I trust that we will be able to devote some time to this aspect of the problem before this conference adjourns.

The only other comments I wish to make concern Dr. Smith's paper on the treatment of fibrositis. He reported that "53 of 60 patients, or 88.3 per cent, were considered as responding well to pregnenolone therapy." I wish I knew what primary fibrositis is. I have often said that God only knows and He will not tell. I personally believe that many of the patients labeled as having fibrositis are individuals suffering from psychoneuroses with symptoms referable to the skeletal system. We have never succeeded in demonstrating any evidence of inflammation in biopsy specimens consisting of skin, subcutaneous tissue, fascia, and muscle, and therefore concluded that the symptoms are not due to inflammation of fibrous tissue. If they are not, then the term "fibrositis" is a misnomer. On the basis of our experience I am of the opinion that so-called "primary fibrositis" is a nebulous term which lacks accurate definition. Secondary fibrositis is real and can be demonstrated microscopically and in the case of rheumatoid arthritis accounts for such symptoms as stiffness and aching. Feeling as I do, I would hesitate to judge the therapeutic efficacy of any of the 11-desoxy steroids as an antirheumatic agent in patients with so-called "primary fibrositis." Improvement in many instances would have to be judged for the most part on the basis of subjective improvement which in the opinion of the American Rheumatism Association Committee "are considered unreliable." As previously stated, the fibrositic component of rheumatoid arthritis is responsible for definite symptoms. These symptoms are relieved promptly by agents possessing antirheumatic activity. This has been demonstrated by the administration of both cortisone and ACTH. I wonder how many of Dr. Smith's patients had rheumatoid arthritis.

R. T. Smith: None of these patients had rheumatoid arthritis. I tried to point out that patients were included in the study only when they failed to show any signs of symptomatic arthritis. I realize that the term "fibrositis" has been a very nebulous entity. For many years we have tried to find pathologic changes that we can call fibrositis. One person finds a pathologic pattern, the next person does not. There has been no specific picture.

I didn't spend much time pointing out what I meant by the fact that fibrositis is due to muscle atrophy and is a provable fact. It is prov-

able if one considers a group of patients suffering primarily from fibrositis. They might have a mild amount of rheumatoid arthritis or osteoarthritis, or some other form of rheumatic disease, but if their symptoms are aching pain and stiffness after inactivity, regardless of what else might be present (provided it is minimal), they can be completely relieved of those symptoms by rehabilitating the muscles. In searching into the history we can find evidence of muscle atrophy due to disuse, decreased use, or strain. There are excellent examples of this in the type which in Philadelphia we designate as the "tired businessman's syndrome." It is not that he is tired because he does too much but because he has done too little. This occurs when a person gets to the age of 45 or 50 years of age. He may have worked very hard during his early years and had a great deal of

FIG. 3. Diagrammatic Representation of Symptoms and Signs in Rheumatoid Arthritis. J = Joint. S = Systemic. F = Fibrositic. P = Psychogenic.

physical activity but as soon as he gets to the place where he can afford to hire someone to cut his lawn or put a motor on his rowboat, etc., and substitutes nothing for that physical activity, he begins gradually over a period of 12 to 24 months to get aching in his muscles and around his joints. If you give that man a simple rehabilitation program within the limit of his tolerance level for fatigue, he will completely rid himself of those symptoms without medication of any kind. We have done that many many times.

On this basis I disagree with the idea that if something helps cure fibrositis, it will aid the fibrositic symptoms of a particular form of arthritis. In evaluating a patient in regard to the fibrositic symptoms we must consider the main features that are involved in a patient with rheumatoid arthritis. There is joint involvement, systemic involvement, fibrositic involvement and a psychogenic involvement. The circle in Fig. 3 represents a particular case in which the joint involvement is quite apparent and very severe; the systemic involvement is extensive with decreased hemoglobin, low red cell count, poor nutrition, and loss of weight; there is quite a bit of fibrositic stiffness and aching; and there exists a large psy-

chogenic involvement. We can see that these three features, the psychogenic, the joint, and the systemic involvement, overshadow the fibrositic. This is the type of case in which pregnenolone would be of no value. Fig. 4 is an example of a patient with minimal joint involvement, minimal systemic involvement, and not too much of the psychogenic involvement, but with a great deal of the aching pain, and stiffness of fibrositis.

FIG. 4. Diagrammatic Representation of Symptoms and Signs in Rheumatoid Arthritis. J = Joint. S = Systemic. F = Fibrositic. P = Psychogenic.

FIG. 5. Diagrammatic Representation of Symptoms and Signs in Fibrositis. J = Joint. S = Systemic. F = Fibrositic. P = Psychogenic.

In this individual there would be overall benefit from pregnenolone. The next diagram (Fig. 5) illustrates a patient suffering principally from fibrositis. There is practically no joint involvement except a limitation in the function due to aching and stiffness with little or no systemic involvement and an average psychogenic involvement. These patients are the types that we chose for our study and they got better with pregnenolone.

The fact that cortisone relieves fibrositis immediately is a very evi-

dent feature but at the same time, if these patients continue to exercise past the tolerance level for fatigue, in spite of cortisone, and in spite of ACTH, they get stiff and achy. I think that this fits right into the premise that we set up this morning. I do not believe that any of the steroids under discussion can overcome the inflammation and the specific involvement of rheumatoid arthritis when that involvement predominates, but they should overcome the main disabling symptoms if fibrositis predominates.

J. Robles Gil: I want to ask Dr. Hellbaum what percentage of the patients with negative results became worse, and also what was the degree of the improvement of the patients with positive results. The other question I want to ask Dr. Hellbaum concerns the stage of the disease in his patients. I think in order to determine the usefulness of pregnenolone and other steroids, it is necessary to establish the stage of the disease as well as the grade of improvement and its duration. Also, it is very important to try to establish or use uniform criteria to judge the grade of improvement. If that is done, it is easy to compare results and decide whether or not a particular steroid is useful.

With respect to Dr. Freeman's remarks, we had two patients who did very well with pregnenolone. They were children about 7 years and 12 years old and they showed by far the best responses to this steroid; I am in agreement with Dr. Freeman's thoughts about the age factor. Since I am going to read my paper tomorrow, I am not going to report the results today. I want to say only with respect to Dr. Hellbaum's report, in connection with sedimentation rates, I had exactly the same experience; I have not observed any constant changes in the sedimentation rate in those patients treated with pregnenolone, even if they get a little bit better.

L. G. F. Nogues: In order that the conference may have all available information on treatment of rheumatoid arthritis with pregnenolone, I want to report very briefly on our experience with this steroid at Bogota, Colombia. We began using pregnenolone three months ago, and up to now we have treated 21 patients. The diagnosis in all our patients was definitely rheumatoid arthritis, as concluded after careful clinical, radiological, and laboratory tests (sedimentation rate, etc.). We found that the most reliable criteria for diagnosis was the radiological one, and all our patients were routinely studied with x-ray pictures of at least both hands, with and without intensifying screens. We did this because we wanted to be certain that we were really treating cases of rheumatoid arthritis. After hearing Dr. Smith's paper, in which his review of 12 so-called cases of rheumatoid arthritis showed that a great many of them were other clinical conditions, we cannot afford to be lax in our diagnostic procedures. I insist on this, because it is a prerequisite for the evaluation of pregnenolone in this disease.

Our patients were in different stages of the disease; the data will be

published later so I do not want to use up valuable time now. All were put on daily oral doses of 300 mg. of pregnenolone. After the first week of treatment we noticed in nearly every case that the sedimentation rate (Westergren) was higher than initially. Due to this finding we pushed the dose up to 600 mg. daily. A week was, according to our experience, a sufficient time interval to determine which patients would have a favorable response to pregnenolone. More or less following the criteria used by Dr. Smith, we have observed a marked grade of improvement of approximately 47 per cent. In all our other patients, treatment was useless.

Now, to finish, I warmly endorse what Dr. Robles Gil has just said about the need for definite criteria to assess the state of the disease in every case. Even more, I think it is quite necessary to have for our use all over the world specific criteria for the diagnosis and evaluation of rheumatoid arthritis. We have to have a common language so that we may be able to understand each other clearly and easily. Something like what the American Heart Association has done in the field of heart disease. If this conference were capable of doing this, I am sure it would be an outstanding contribution to the study of so-called "rheumatic disease."

F. Homburger: I should like to ask Dr. Hellbaum a specific question and to make some general remarks. Regarding the effects observed by Dr. Hellbaum in those patients receiving testosterone propionate for long periods of time, I should like to raise the question whether the improvement he reported might not have been due to the effects of testosterone upon osteoporosis rather than to a true anti-inflammatory effect of that drug. Likewise, I should like to advance an explanation for the beneficial effects of pregnenolone when given during long periods of time, particularly with respect to the antifatigue and other possible nonspecific effects of that compound rather than a true antiarthritic activity in the narrow and specific sense of that word. In our experience, which is practically limited to older people, we have found osteoporosis in practically all cases. When good effects have been obtained with ACTH or cortisone in the treatment of the arthritis in such individuals, there often remain severely incapacitating pains in the muscle insertions and bones, due to osteoporosis, which prevent the patients from fully using the newly won freedom of motion of the joints. Such pains will respond in three months or more to high doses of testosterone, preferably given in combination with estrogens and, according to suggestions by Dr. E. Shorr of the New York Hospital, in combination with vitamin D and strontium lactate. This therapy has to be continued indefinitely and, while symptomatic improvement of osteoporotic pain will occur in a few months, x-ray evidence of ossification may not be expected in less than one year. In order to avoid undesirable virilization in women so treated, we have attempted to use methylandrostenediol, which we had previously found to be less virilizing than testosterone. Some caution may be indicated in the use of this new drug in arthritic patients since we have observed acute exacerbations in two patients receiving methylandrostenediol to promote

bone calcification. Whether these exacerbations were indeed due to the drug is of course impossible to say at this time.

Now, I should like to philosophize along the lines previously mentioned by Dr. Bauer, namely on methods of evaluation of steroids and other substances which may be "antiarthritic" to a lesser degree than cortisone and ACTH. Those of us interested in the chemotherapy of cancer have long been acquainted with the difficulties of such evaluations, which may be new to the rheumatologists since previously they never had to deal with relatively effective "antirheumatic" agents. In cancer, chemotherapy requirements for evaluation have become fairly well standardized. We insist that the disease be proved by biopsy; we take into account the natural course of the disease, and we are well aware that besides the nonspecific factors known to alter the course of the disease, there are others which we do not know that may play a role. In arthritis we do not seem to deal with a well-defined disease but rather with a symptom complex and in order to arrive at comparable series of patients, we must define more exactly the type of case that may be included in a series of patients studied for a comparative evaluation of the "antiarthritic" property of new substances. This alone would be a great contribution to rheumatology. In cancer of the prostate or in leukemia we have at least measurable characteristics to judge the course of the disease. These are lacking in arthritis, since it seems quite certain that neither the sedimentation rate nor the electrophoretic patterns of the plasma protein, to mention only two possible approaches to this problem, bear any relation to the clinical course of arthritis. In spite of this, there seem to be already available means to improve our evaluation of such experimental therapeutic series which would tend to render results more comparable. We know from Dr. Freeman's paper that there is a nonspecific psychologic effect of any new medication which accounts for as many as 50 per cent of the improved patients when placebos are being given. Cinematographic records before, during, and after therapy provide a means to measure and record responses of individual joints, and there are standards of reference, such as cortisone, to express intensity of improvement. Thus if we adopted a standardization of cases, a uniform evaluation by the best objective means possible, and the use of placebos as well as known active antirheumatic substances in addition to the unknown substances in every patient tested, our results would become at once much more valuable, and reliable information would be gained more quickly.

A. Hellbaum: I would like to answer Dr. Homburger's question regarding osteoporosis first. All of the patients were subjected routinely to x-ray in the diagnostic procedure for the juxta-articular demineralization, characteristic of rheumatoid arthritis. Testosterone, even over long periods of time, did not improve the osteoporotic changes as might have been anticipated. However, noticeable improvement occurred in some patients.

I am sorry I cannot give the exact percentage of patients who became

worse with pregnenolone, but there were a number. In the first series, therapy was discontinued in 11 patients because of side-effects but only in one patient in the second series.

I agree that we need more uniform criteria for the evaluation of patients. We also need more satisfactory objective tests. Those we now use do not indicate adequately the status of the patient, and have little prognostic value. Perhaps we should broaden our horizon somewhat. I question whether rheumatoid arthritis is an adrenal disease or even an endocrine disease although it may involve endocrine glands. I am certain that Dr. Selye will agree that the adaptation syndrome is concerned with more than the adrenal-pituitary axis. Every tissue and organ in the body responds to a severe traumatic incident. If we knew enough about cytologic aspects of the liver, perhaps we would also find marked and dramatic morphologic changes following severe traumatic insult.

In thinking of objective tests, I am reminded of a conference which was called for the purpose of establishing more satisfactory criteria for choosing medical students. For several days, many "objective" tests were presented. Finally a naval officer spoke up, stating that it was his job to choose combat pilots. He had at his command many "objective" tests, each as good as the interpretation of the man designing the test. There was some degree of correlation between some of the tests and progress in early preflight training, but as the training program continued the correlation became less and less. Finally, in actual combat there was no correlation at all. Thus, in choosing objective tests, let us be certain that they truly indicate the prognostic status of the patient over a prolonged period of time, and, when used for screening new compounds, that the tests represent the condition for which we are screening.

E. B. Astwood: It might be pertinent to cite briefly the experience with these compounds obtained in our hospital. My associates, Drs. Payne and Raben, and I have been chiefly concerned with an attempt to purify ACTH (adrenocorticotrophin). As a by-product of this study we have had available ample quantities of ACTH for clinical use and with Drs. Rosenberg and Cleroux we have treated 211 patients, including 55 with rheumatoid arthritis. Concurrently, Drs. Brugsch and Manning carried out a study with pregnenolone and acetoxypregnenolone in 22 patients and it was possible to compare the two types of treatment.

Sixteen patients were treated with pregnenolone for four to eight weeks and six with acetoxypregnenolone for two to four weeks, the dosages ranging from 100 to 1200 mg. a day by mouth or by intramuscular injection. All of the cases were classified as stage 2 or stage 3 except one who was in stage 4. The observations showed that, in agreement with what has already been said, the eosinophils did not fall; indeed they tended to rise, especially when the material was given by injection. There was a tendency for the total ketosteroid excretion to fall and after treatment for some days there appeared in the urine material which we took to be pregnanediol. Uric acid excretion and the uric acid-creatinine ratio did

not change nor was the blood uric acid concentration affected. The body weight did not change. The sedimentation rate slowly declined in one case. This one patient showed both objective and subjective improvement. In the other 21 cases, however, there was no change in the sedimentation rate, nor was there any clear objective improvement. There was no change in the anemia nor in the size of rheumatic nodules. Nine of the patients were subsequently treated with ACTH, with the usual results. On the bases of these observations it was our conclusion that these two compounds, in the way in which they were used, were not effective, but I should add that this conclusion may have been colored by the fact that other patients were being treated concurrently with ACTH, where the response was so impressive that our criteria may not have been proper, and minor degrees of improvement may not have been so classified.

A. White: In listening to these papers and to the discussion which has transpired, some thoughts occur to me which lead to several questions, three in number, two of which might perhaps be addressed to the audience in general, and one to Dr. Hellbaum. A good deal has been said about the sedimentation rate and I should like to comment briefly on the relationship of this index to the plasma protein pattern. I think it is generally agreed that in circumstances in which ACTH and cortisone have been used clinically and in which there is an abnormal albumin to globulin relationship, that this relationship is altered in the direction of normal, concomittant with therapeutic benefit to the patient and a paralleling return of the sedimentation rate to normal. We have heard in the papers presented, and in the discussions, that in therapy with the 11-desoxy compounds, there apparently is no alteration in at least one of the therapeutic indices, namely, the sedimentation rate. I should like to remind you that some years ago it was demonstrated that the fraction of the serum proteins which appeared to be concerned with variations in the sedimentation constant was the α globulin fraction. I point this out because recently in California, Dr. Roberts in our laboratory has been examining some of the physiologic effects of one of the desoxy steroids, namely, Compound L, and one of the most striking observations is that following the administration of Compound L to rats for a period of eight days, in a daily dose of 10 mg. per rat, there is a very significant increase in the α_1 globulin fraction of the serum proteins. Our first thought was that this was perhaps the response to injury since the insolubility of Compound L may create certain discomfort in the animal. However, I should also like to remind you that the plasma protein alteration in injury, as exemplified in the work of Chanutin and his colleagues with turpentine, burns, and nitrogen mustard administration in rats, is in the α_2 globulin of the serum. I should like, therefore, to pose the general question of whether anyone has examined possible alterations in the serum or plasma protein pattern of individuals treated for long periods of time with pregnenolone. If 11-desoxy steroids in general elevate

the α globulin fraction, this might account for the failure of the sedimentation rate to alter with therapeutic trials of these compounds.

The other general question I should like to pose is the possible relationship of thyroid activity of patients who have been presented this morning to their responsiveness to various types of medication. Now, it seems to become increasingly clear, as has been known for a long time from animal experimentation, that in circumstances of depressed activity of the thyroid, the degree of tissue response to various types of medication is diminished. In human studies, individuals such as Dr. Thorn are beginning to talk about "myxedema" of the adrenal cortex, that is to say the lowered response of the adrenal cortex to ACTH in individuals with hypothyroidism. Also, tissue effectiveness in interconversion of certain steroids, or other steroidal transformations, may be diminished in hypothyroidism. Moreover, we have been discussing this morning patients in whom there may be malnutrition, in which condition there may be lowered thyroid function as well as other endocrine inadequacies. This could be one of the variables in terms of responsiveness of the tissues to various types of medication.

Finally, I should like to ask Dr. Hellbaum about a statement which he made which has important implications because it touches upon the fundamental problem of the possible mechanism of action of steroids. Dr. Hellbaum, I believe you said that on the basis of your experience with testosterone and pregnenolone you believe that the effectiveness of these compounds, in the instances in which they had proved to be therapeutically active, was an indirect metabolic effect rather than a specific one. Will you develop this point a little more?

A. Hellbaum: My answer, Dr. White, has to be somewhat in the realm of speculation. Perhaps it is time that we think of these steroid compounds as chemical substances capable of producing physiologic alterations other than their specific hormonal influence. Hormonal substances have been used in certain conditions because they are more readily available, but the beneficial effects may be independent of the "hormone" activity as we know it. Androgens and estrogens are more than mere "male" and "female" hormones. When one considers the influence on maturation and growth, nitrogen retention and protein metabolism, electrolyte and fluid balance, cardiovascular system, hematopoiesis, bone mineralization, and increase in appetite, weight gain, and strength, then the "sex" phase or activity becomes somewhat secondary.

It was suggested this morning that the beneficial effects of new compounds be evaluated in terms of the patient's response to cortisone. This may be satisfactory if we keep in mind that the mechanism of action of the various substances, as well as the type and duration of response, may be different and not comparable. We know that cortisone has an "anti-inflammatory" action as manifested by its effect, not only in joints, but in allergic states and inflammatory conditions of such structures as the eye. Thus, since the joint involvement may be but a peripheral manifesta-

tion of some underlying metabolic alteration, does cortisone influence the underlying cause or merely benefit a peripheral symptom?

I doubt that testosterone or pregnenolone have the same mechanism of action. If so, they would be efficacious in the same patients. They are not. We do not advocate that testosterone or pregnenolone are the compounds of choice for the treatment of rheumatoid arthritis. But the same may be said for the other steroids which have been investigated to date. The answer may not even be in the steroid field. Hence, continued search must be made for substances which may exert a greater and more lasting beneficial response with a decrease in the undesirable manifestations.

F. Homburger: Dr. Bernfeld in our laboratory has studied the electrophoretic patterns in the blood plasma of 47 patients with arthritis and other diseases who received ACTH and in some patients while they were given pregnenolone. These studies were done during long periods of time, at weekly or more frequent intervals, sometimes hours, after as little as 25 mg. of ACTH Armour Standard had been given. He found that the only plasma protein that showed changes was the β globulin (with the exception of one case of pemphigus where there were changes in the γ globulin). Such changes occurred in only 15 per cent of all the patients studied. Sometimes there was a drop of the β globulin during administration of ACTH and sometimes (often in the same patients) there occurred a marked rise following discontinuing therapy. In one case of Still's disease in a youngster, the β globulin reached amounts such as are only seen in multiple myeloma. The patients receiving pregnenolone showed no plasma protein changes.

T. S. Danowski: In three of the small group of patients that we have been able to study rather intensively we found lowered levels of serum-bound protein iodine prior to pregnenolone therapy. These were not the result of lowered serum albumin values, which may be associated with lowered levels of protein-bound iodine. Curiously enough, these three patients fall into the group of patients who might have had some improvement from pregnenolone, so that from the point of view of Dr. White's suggestion this would be a contrary finding. I would also like to point out that characteristically, following the administration of cortisone or ACTH, there occurs a drop in protein-bound iodine and in the uptake of radioactive iodine, so that presumably these compounds are producing clinically beneficial effects in the face of decreased thyroid function. Of course, a decrease in iodine uptake or decreases in circulating protein-bound iodine could be present with perfectly normal concentrations of thyroxin or thyroid hormone in the peripheral tissues at the time cortisone and ACTH were acting.

R. H. Freyberg: I would like to speak on three points. I wish to emphasize that in judging results of substances being tested for antirheumatic effect, it is important whether or not the persons judging these have had oppor-

tunity to compare them with the effects of cortisone and ACTH. The changes that come from cortisone and ACTH administered to patients with rheumatoid arthritis are characterized by three features: (1) the *speed* with which the effects occur; (2) the *high magnitude* of the result, and (3) the *almost consistent* response that is obtained. In evaluating an X substance, I think that the end evaluation may be different whether or not it is compared with cortisone or ACTH. Whether or not this comparison is made may explain some of the differences that are seen in the literature in regard to evaluation of X substances. Whether or not it explains any differences in reports presented at this conference I do not know.

Second, in the reports this morning it was mentioned that we needed to have a long period of administration in order to evaluate some of these steroids. I am confused as to why that is emphasized, because in most of these reports where benefits were reported, they were noted usually within the first or second week, and many substances were screened for from only one to three weeks.

Third, a question to Dr. Davison. He used several preparations of pregnenolone and I would like to know what, in his judgment, is the relative effectiveness of each.

R. A. Davison: Dr. Bauer showed a typical response of patients suffering from rheumatoid arthritis following the use of cortisone or ACTH. That typical response is very dramatic, and sedimentation rates came down. I have compared pregnenolone with results that we obtained using cortisone or ACTH after the use of pregnenolone. We have also given patients cortisone or ACTH who have not had pregnenolone. Unfortunately we do not get 100 per cent results with cortisone or ACTH. We have several patients in whom cortisone or ACTH has given little remission; patients who have received cortisone for a period of six or seven months who still have increased sedimentation rates. In one of the failures reported in this series with pregnenolone, we had a dramatic improvement clinically with cortisone but the sedimentation rate did not fall. However, we have had enough failures with cortisone to believe that while it is a very valuable drug, in the patients who have obtained remissions, the dosages were high and produced effects which were undesirable. I have in mind two patients who have been on our ward four months who have gotten excellent results with the relief of arthritic changes. Both of these patients have a definite Cushing's syndrome, and one of them has impaired dextrose tolerance. We have not observed definite changes in plasma protein in the patients in whom these determinations have been made.

With regard to the different preparations of pregnenolone, my early feeling was that our best results came from the use of pregnenolone acetate in sesame oil. This material was difficult to handle and we obtained a number of local tissue reactions so that I felt it was unwise to continue this type of medication. The results with crystalline pregnenolone in aqueous suspension are not as good as they had been with the oily

material. Just why, I do not know. Pregnenolone acetate orally, in my experience, certainly is not as good as the material given by injection. But the disadvantage of injection of large doses of material which is poorly absorbed is one of the things which limits its usefulness.

A. R. Abarbanel: I'd like to expand just a bit further on the question of thyroid activity and the overall effects of steroids. Many years ago we had a small number of patients in the climacteric who did not respond to estrogens until they had been given thyroid extract sufficiently great to improve their metabolic rate to or toward normal. Tomorrow we will show that with pregnenolone there is usually no response until the basal metabolic rate is normal. Further, particularly in Southern California, where it is warm, we have found that response to thyroid extract is unpredictable and that by far the most consistent and persistent results are obtained with the use of thyroxin. I would like to have some help on this score, as it puzzles me.

As far as the psychosomatic aspects of the problem are concerned, I do not think that we are solving anything by giving the patient just a placebo. We can only solve this situation, in part at least, by finding out a bit more about the patient—her home life, economic and social atmosphere, etc. What is it that makes her get worse? For example, if the patient has a daughter who is 16 and comes in late, the mother's arthritis is worse the next day. And so we must evaluate not the symptoms but the patient with the symptoms. At least 30 per cent of all your patients will get better because you tell them they are going to get better. The power of suggestion alone is adequate.

With my meager knowledge of the field of rheumatology, I do not feel that subdeltoid bursitis should be included as a test object for steroid therapy. In some work with iontophoresis, my colleague, Dr. George O. Shecter, has found that in 76 cases of acute subdeltoid bursitis it was possible to get adequate relief in 74 cases in between 48 and 72 hours by merely ionizing magnesium ions directly into the bursa. I am wondering of what value physiotherapy, from the viewpoint of heat, or ionizing substances such as histamine, the steroids, or magnesium into a definite area would have? It might bring relief and then steroid therapy might maintain that relief. In other words, could we use that as another adjuvant in the management of the patient?

T. H. McGavack: In connection with Dr. Abarbanel's comments, if we use our placebo with the same interest and enthusiasm that we use our drugs, we eliminate some of the psychic effects that he mentioned. And, if our workers are not aware whether they are using the drug or the placebo, they are quite likely to exhibit this enthusiasm. Also, our patients have to be treated as individuals, at least from an endocrine standpoint, so that if we are doing the best for the patient we may often combine several endocrine therapies. In screening a new drug, however, it seems

that we should if possible give it alone until we know something of its own action.

I have three questions. Has Dr. Hellbaum seen a difference in the response to the osteoporosis in relation to the amount of joint motion attained? A question for Dr. Davison; in one of the cases he described he mentioned the appearance of psychosis during the use of pregnenolone therapy. Was this a coincidental or a causal relationship? A question to Dr. Bauer; how many of the 13 patients had had ACTH or cortisone previously or concomitantly with pregnenolone therapy?

I. T. Nathanson: I should like to amplify the point raised by Dr. McGavack, namely, that for proper evaluation of a new compound, the substance should be tried first in untreated patients. This has been given utmost consideration in our screening program on the effects of steroids in cancer patients. The response of an individual to a compound may be modified considerably if it is tested subsequently to a substance that has an obvious metabolic and clinical effect. Moreover, it is problematical whether a patient not obviously responding to an initial test compound can be considered in the same class as an untreated patient when submitted to trial with a second substance. An apparently inert compound may possess subtle properties that may alter the physiologic status of the individual without being detected. Hence, it is possible that under these circumstances as well, the response of a patient to a second compound may be different than if it had been originally employed. Evidence of these points has been accumulated in studying the effects of various steroids on susceptible types of cancer.

W. Bauer: No one of the 13 patients treated with oral pregnenolone (Table I) received either cortisone or ACTH concomitantly or prior to the institution of pregnenolone. The two patients who received pregnenolone intramuscularly subsequent to cortisone are not included in Table I.

R. A. Davison: In answering the question of Dr. McGavack, with respect to the particular patient who developed a psychosis; I feel certain it was not due to pregnenolone administration. He had had a psychotic episode two years prior to any of the steroid hormone medication.

A. Hellbaum: I cannot answer your question, Dr. McGavack. At the present time we cannot evaluate the relationship of the degree of osteoporosis to joint mobility in rheumatoid arthritis.

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Experiences with Pregnenolone and Other Steroids in Patients with Rheumatoid Arthritis*

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This report will discuss only clinical experiences with various steroid preparations administered to patients with rheumatoid arthritis. The studies were done in the Research Unit of the Hospital for Special Surgery in connection with the Department of Internal Medicine, Cornell University Medical College, New York. Only through the co-operation of many persons on the clinical and research staffs and the several pharmaceutical manufacturers who generously supplied the steroid preparations could these studies be made. Our appreciation is hereby extended to these individuals.

The investigations to be discussed were always conducted in patients who had been studied carefully, especially with regard to diagnosis, and whose disease course had been followed for long periods, generally for several years. The usual procedure was to administer the steroid to be screened either before or after the use of cortisone or ACTH so that the effects of the test preparations could be compared with cortisone or ACTH. Except as otherwise stated, the steroids tested were given intramuscularly.

Results

The results can be summarized best in tabulations. The effects of many preparations screened are shown in Table I. *Pregnenetriolone* (Δ^4-pregnene, 17α, 20β, 21-triol, 3-one) was injected into two male and two female patients (Table I, Section A). The arthritis in these cases was

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TABLE I

CLINICAL TESTS OF VARIOUS STEROIDS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Medication	Patients						Response				
Nature	Days	Total Amount, Gm.	Sex		Age	Stage	Functional Classification	Clinical Subjective	Clinical Objective	E.S.R.*	Complications
A	14	2.085	W.H.	M.	41	II	II	+	+	0	None
	5	1.175	F.S.	F.	28	II	II	0	0	I	Local tenderness
	12	1.600	R.E.	M.	56	III	III	0	0	I	None
Pregnenetriolone (Δ^4-pregnene, 17α, 20β, 21-triol, 3-one)	4	0.900	B.E.	F.	26	II	II	0	0	I	Local tenderness
B	9	1.600	E.H.	F.	40	II	II	0	0	0	Dizziness, headache
	11	2.085	J.B.	F.	49	III	III	0	0	0	None
	6	1.200	R.E.	M.	56	III	IV	0	0	I	None
Pregnenetriolone diacetate											

*In the column showing E.S.R. response, the letter "I" means increase, and "0" indicates no significant change.

TABLE I—(*Continued*)

Medication		Patients					Response				
Nature	Days	Total Amount, Gm.	Sex		Age	Stage	Functional Classification	Clinical Subjective	Clinical Objective	E.S.R.	Complications
C 3, 21-Dihydroxy, 11-keto, Δ^{17}, 20-cyanopregnene	17	2.020	J.R.	F.	49	IV	IV	0	0	0	Induration and abscess
	3	0.285	N.M.	F.	59	II	II	0	0	0	Local tenderness and induration
D Dihydrocortisone	2	0.400	L.C.	F.	52	II	II	0	0	0	Febrile episode
	12	1.475	J.R.	F.	49	IV	IV	+	0	0	Local tenderness
	6	0.420	J.L.	M.	43	IV	IV	+	0	0	Chest pain
	9	1.150	J.C.	M.	54	II	III	0	0	0	Severe local tenderness
	7	0.375	F.S.	M.	76	II	II	0	0	0	Local tenderness

E	11	2.350	M.H.	F.	39	II	II	0	0	0	None
	12	2.750	E.N.	F.	50	II	II	0	0	0	Local induration and tenderness
F	20	3.800	M.K.	F.	31	II	I	0	0	0	None
	11	2.050	M.H.	F.	39	II	II	0	0	0	None
	8	1.185	J.C.	M.	54	II	III	0	0	0	None

17-Hydroxyprogesterone

Reichstein's S acetate (17α-hydroxy, 11-desoxycorticosterone, 21-acetate)

TABLE I—*(Continued)*

Medication		Patients					Response				
Nature	*Days*	*Total Amount, Gm.*	*Sex*		*Age*	*Stage*	*Functional Classification*	*Clinical Subjective*	*Clinical Objective*	*E.S.R.*	*Complications*
G	8	1.305	M.S.	F.	31	III	II	0	0	0	None
	4	0.760	M.F.	F.	47	III	II	0	0	0	None
	7	0.875	M.H.	F.	39	II	II	0	0	0	None
Δ^5, 17-Ethinylandrostene, 3, 17-diol											
H	8	1.385	M.J.	F.	59	IV	IV	0	0	0	None
	12	1.785	M.F.	F.	47	III	II	0	0	0	None
	6	1.085	J.B.	F.	49	III	III	0	0	0	None
Δ^4-Androstene, 3, 17-dione											

I	12	2.585	R.E.	M.	56	III	III	+	0	0	None
	8	1.200	P.G.	M.	56	III	III	0	0	0	None
	7	1.050	K.L.	F.	53	II	III	0	0	I	None
	7	1.000	C.W.	F.	60	II	II	+	+	0	None
	7	1.000	L.D.	F.	54	II	III	0	0	0	None
	9	1.685	C.R.	F.	64	III	III	0	0	I	Local induration
J	16	3.000	H.R.	M.	52	III	III	0	0	0	None
	14	2.100	J.C.	M.	54	II	III	0	Worse	0	Elevation of temperature and pulse

21-ol, 3, 20-dione, Allopregnane, 21-acetate

Testosterone

TABLE I—(*Concluded*)

Medication		Patients						Response			
Nature	*Days*	*Total Amount, Gm.*	*Sex*	*Age*	*Stage*	*Functional Classification*	*Clinical Subjective*	*Clinical Objective*	*E.S.R.*	*Complications*
K Estradiol	7	0.0023	E.S.	F.	52	II	III	0	0	0	None
	3	0.0028	J.C.	M.	54	II	III	0	0	0	None
L Δ^5, 3-Acetoxyetiocholenic acid	16	1.985	W.D.	M.		III	III	+++	++	0	Local tenderness and induration

potentially reversible (i.e. the disease was stage II or III, American Rheumatism Association classification). The clinical response was very slight (+) or none. The sedimentation rates did not improve in any of the patients. In two patients the steroid preparation produced discomfort at the site of injection. It was considered to have no significant therapeutic effect. The *diacetate* of this preparation (Table I, B) was studied in one male and in two female patients, all of whom were in a stage of disease that would have made possible ready recognition of any effects. No subjective or objective improvement occurred and there was no im-

FIG. 1. The clinical course of a patient to whom pregnenetriolone diacetate was administered. The drugs administered are charted at the top, the clinical changes are shown in the panel next to the top, and other effects are plotted in the lower sections of the graph.

provement in sedimentation rates. Obviously, this preparation, also, had no therapeutic effect.

The course of events in one patient who received pregnenetriolone diacetate is illustrated in Fig. 1. After a period of ACTH administration with improvement in the rheumatic disease, 200 mg. of pregnenetriolone diacetate were given daily. The rheumatic state became progressively worse, and there was an increase in the sedimentation rate. With subsequent use of ACTH, improvement again occurred. The lack of effect of pregnenetriolone is thus clearly shown.

In section C, Table I, are tabulated the effects of *3, 21-dihydroxy, 11-keto,* Δ^{17}*, 20-cyanopregnene*. This steroid was found to be of no value

as judged from administration in two female patients. It caused local induration in both patients, and an abscess in one. Because of these local undesirable effects, studies of this steroid were discontinued.

Dihydrocortisone, a saturated steroid, was tested in two female and three male patients (Table I, D). No therapeutic effect was observed when the compound was used in doses of 100 to 200 mg. daily. Various undesirable side-effects were noted in all patients.

Injections of *17-hydroxyprogesterone* were administered to two female patients in doses up to 300 mg. daily (average of 225 mg. daily). No effect of any type was observed except tenderness and induration at the site of injection in one patient (Table I, E).

We were very much interested in Reichstein's *Compound S acetate* (17α-hydroxy, 11-desoxycorticosterone, 21-acetate). In two female patients and in one male patient whose arthritis was early enough to be readily relieved by therapeutically effective substances, no effects were observed with doses up to 300 mg. a day. One patient (M. K.) also had moderately severe diabetes mellitus, which was uninfluenced during the three weeks that the steroid was used (Table I, F).

Δ^5, *17-Ethinylandrostene, 3, 17-diol* was given to three female patients in doses of 100 to 200 mg. daily and produced no change in the arthritis (Table I, G). Similar negative results were obtained with Δ^4*-androstene, 3, 17-dione* in dosage up to 200 mg. daily, given to three patients (Table I, H).

Allopregnane acetate (21-ol, 3, 20-dione, 21-acetate), supplied to us through the courtesy of Dr. George Farrar, was injected into four female and two male patients, in dosages up to 300 mg. daily. In four patients there was no effect; in two patients, only a very slight improvement, chiefly symptomatic, was noted. Sedimentation rate did not improve; in two patients, it increased. Usually the drug was tolerated well (Table I, I).

Two examples of the lack of effect of *testosterone,* given to 12 patients, and of *estradiol,* given to six patients, are shown in sections J and K, Table I. No beneficial effect was observed even with quite large doses. Δ^5, *3-Acetoxy-etiocholenic acid* was available in only a small amount, sufficient for testing in a single male patient. It was injected in doses up to 200 mg. daily, usually 100 mg. daily. Significant subjective and objective improvement occurred although there was no change in the sedimentation rate (Table I, L). The sites of injection became tender and indurated. There are plans to study this steroid further.

Pregnenolone and 21-Acetoxypregnenolone. Various preparations of pregnenolone and 21-acetoxypregnenolone were studied. The results are summarized in Table II. Our earlier experiences were with acetoxypregnenolone, which was injected in doses of 100 to 400 mg. daily in nine patients for from 14 to 42 days. It was tolerated well but in only three patients was there improvement, and this was moderate and only symptomatic. Pregnenolone and pregnenolone acetate were injected intramuscularly into 10 patients in doses from 300 to 400 mg. daily. Slight subjective

TABLE II

EXPERIENCES WITH PREGNENOLONE PREPARATIONS AND WITH 21-ACETOXYPREGNENOLONE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Preparation	Daily Dose, Mg.	Number of Cases	Results: Worse	Results: No Change	Results: Slight Improvement	Results: Moderate Improvement	Days Treated	Complications
21-Acetoxypregnenolone (Intramuscular)	100 to 400	9	1	5	0	3	14 to 42	
Pregnenolone and pregnenolone acetate (Intramuscular)	300 to 400	10	4	4	2	0	16 to 30	Painful injection, 2 Edema, 1
Pregnenolone acetate (Oral)	300 to 850	44	13	14	8	9	17 to 104	Edema, 5 Menstrual irregularity, 3 Wakeful and jittery, 1

improvement occurred in only two patients. Injections produced considerable pain in two patients; edema developed in one.

If a steroid is to be used for long periods in ambulatory patients there is a great advantage in using oral preparations if they are effective. Most of our experience was with Δ^5-pregnenolone acetate tablets. Only 9 of 44 patients improved moderately, 8 had slight symptomatic improvement. There were five instances of edema, three of menstrual irregularity, and one patient became considerably nervous and wakeful.

The experiences with oral pregnenolone acetate are now analyzed in more detail. The patients were divided into two groups, one group consisting of those patients who received 400 mg. or less of the steroid daily, the other group made up of those who received 425 to 850 mg. daily. In the group that was given the smaller doses (Table III), there were 6 male and 12 female patients whose ages ranged from 6 to 68 years. In only five patients could any degree of improvement be observed and this was only symptomatic and to a limited degree. Grading response to treatment according to the criteria of the American Rheumatism Association, there was no benefit except in three patients, H.A., G.R., and S.P. who had grade III response (minor improvement). In only one instance, A.R., did the sedimentation rate decrease and this happened in a patient who showed no clinical improvement.

In Table IV are shown the results among the group of patients who received more than 400 mg. of oral pregnenolone acetate daily. There were 7 male and 13 female patients, ranging in age from 27 to 62 years. In all but one patient the disease was in stage I, II, or III (potentially reversible); E.D. had stage IV disease. Treatment was given for from 21 to 71 days. Half of the patients showed no degree of improvement, but various degrees of symptomatic improvement occurred in the remaining half of the patients. The functional classification (according to American Rheumatism Association criteria) showed improvement in only five cases and in only one was this improvement of more than one step (grade IV to grade II in E.D., a patient with severe arthritis, stage IV). According to the American Rheumatism Association therapeutic criteria, improvement, when it occurred, was grade III (minor) in all but patient E.D. in whom it was grade II. In only two of the cases did the sedimentation rate decrease.

Table V presents the results in all patients (regardless of dosages) who received pregnenolone acetate orally. The data are shown in relation to the stage of the arthritis. It should be noted that only three patients (7 per cent) showed a therapeutic response of grade II; none had remission—grade I response. In 14 patients (32 per cent), grade III response (minor improvement) was reported and in 27 patients (61 per cent), response was grade IV (no improvement). Considering those who had any degree of improvement (39 per cent of all patients), this was chiefly minor and symptomatic improvement. In two of the patients showing grade II improvement, the benefit gradually lessened while the treatment was being continued; the steroid was stopped in these cases because both the

TABLE III

INDIVIDUAL RESPONSES AMONG PATIENTS WHO RECEIVED PREGNENOLONE ACETATE (ORALLY) IN DOSES UP TO 400 MG. DAILY

Patients	Sex	Age	Disease Duration Yrs.	Disease Stage	Days	Therapy Daily Dose Mg.	Total Dose Gm.	E.S.R. Change*	Result Subjective Improvement	Functional Before	Classification After	Grade of Response to Therapy (A.R.A. Class)
M.C.	F.	38	12	III	44	300	13.45	0	0	III	III	IV
S.W.	F.	53	3	II	35	350	13.0	I	—	II	III	IV
K.A.	F.	6	3	I	28	375	10.5	0	+	I	I	IV
S.U.	F.	47	20	III	29	400	11.6	0	0	II	II	IV
G.L.	F.	48	5	III	17	400	6.8	0	0	III	III	IV
A.C.	M.	60	6	III	21	400	8.4	0	+	III	III	IV
H.E.	F.	61	25	III	23	400	9.2	0	0	IV	IV	IV
S.C.	F.	41	8	III	25	400	10.0	0	0	III	III	IV
H.A.	M.	45	2	Spond.	33	400	13.7	0	—	IV	IV	IV
N.E.	F.	48	8	II	44	400	14.4	0	—	II	II	IV
F.E.	F.	48	11	III	38	400	17.8	I	—	III	III	IV
W.A.	M.	68	3	IV	52	400	20.5	0	0	IV	IV	IV
A.R.	M.	60	6	III	56	400	21.1	D	0	III	III	IV
W.E.	F.	51	7	II	58	400	23.5	0	0	III	III	IV
A.D.	M.	46	10	II	70	400	27.7	0	0	II	II	IV
S.P.	M.	61	12	III	21	400	8.2	0	++	III	II	III
G.R.	F.	33	13	IV	74	400	29.6	0	++	IV	III	III
H.A.	F.	28	4	II	104	400	41.6	0	+	III	II	III

* The "0" means no significant change, the letter "I" means increase, and "D" means decrease.

TABLE IV

INDIVIDUAL RESPONSES AMONG PATIENTS WHO RECEIVED MORE THAN 400 MG. PREGNENOLONE ACETATE (ORALLY) DAILY

Patients			Disease			Therapy			Result			Grade of Response to Therapy (A.R.A. Class)
	Sex	Age	Duration Yrs.	Stage	Days	Daily Dose Mg.	Total Dose Gm.	E.S.R. Change*	Subjective Improvement	Functional Before	Classification After	
R.U.	F.	62	10	III	49	425	21.7	0	Worse	III	III	IV
H.U.	M.	54	5	III	21	450	9.8	0	0	III	III	IV
R.U.	F.	38	14	III	40	450	18.0	0	+	II	II	III
C.A.	M.	54	7	III	71	475	34.2	0	++	II	II	III
R.O.	F.	55	5	III	57	475	29.0	0	+	III	III	III
D.O.	M.	55	15	III	40	475	18.7	0	++	III	II	III
E.M.	F.	27	9	III	21	500	10.5	0	Worse	III	IV	IV
J.O.	F.	51	23	III	34	500	17.0	0	0	II	II	IV
B.A.	F.	48	13	III	35	500	17.5	0	Worse	II	III	IV
W.Y.	M.	48	17	Spond.	40	500	20.0	I	0	III	III	IV
C.A.	F.	47	1	I	46	500	23.0	I	0	II	II	IV
H.A.	F.	46	4	II	55	500	27.5	I	0	III	III	IV
R.O.	M.	45	8	III	21	500	10.5	0	+	II	II	III
C.O.	F.	42	13	III	28	500	14.0	0	++	III	II	III
H.A.	F.	61	10	III	29	500	14.5	D	++	III	II	III
M.A.	F.	29	5	II	49	500	24.5	0	+	II	II	III
E.D.	M.	56	10	IV	62	500	30.6	D	+++	IV	II	II
S.M.	F.	31	10	III	39	550	23.2	I	Worse	III	III	IV
M.C.	M.	62	15	III	30	650	13.2	0	+	IV	IV	IV
S.C.	F.	28	1	I	31	850	27.3	0	+++	III	II	III

* See footnote to Table III.

TABLE V

THERAPEUTIC RESPONSE TO ORAL PREGNENOLONE ACETATE IN 44 CASES, TABULATED IN RELATION TO THE SEVERITY (STAGE) OF THE DISEASE GRADED ACCORDING TO CRITERIA OF THE AMERICAN RHEUMATISM ASSOCIATION

Stage of Disease	*Number of Cases*	*Grade of Therapeutic Response*			
		I	*II*	*III*	*IV*
I	3	.	.	1	2
II	7	.	.	2	5
III	28	.	2	10	16
IV	4	.	1	1	2
Spondylitis	2	.	.	.	2
Totals	44	.	3	14	27

patients and the doctors considered it no longer of value. In the third patient with grade II improvement, pregnenolone was discontinued and a placebo was substituted; the patient became worse; pregnenolone acetate was given again, exactly as it was originally used, and there was no improvement.

All of our experiences with pregnenolone acetate administered orally are summarized in Table VI. In 61 per cent of the patients there was no improvement (grade IV response). In one-third of the patients there was only minor improvement (grade III response). This was chiefly subjective and symptomatic. Such improvement was seen more often when the dose was higher. In three cases there was significant improvement (grade II response); but this was only temporary in two patients and could not be repeated in one patient. These three patients were in the group of patients who received larger doses of pregnenolone, among whom improvement was noted more often. It should be emphasized again, however, that this improvement was of a minor degree in most cases; it was chiefly subjective and often consisted of improvement in the patient's general sense

TABLE VI

SUMMARY OF EXPERIENCES WITH PREGNENOLONE ACETATE ADMINISTERED ORALLY TO PATIENTS WITH RHEUMATOID ARTHRITIS. CORRELATION TO THE DOSE OF THE STEROID IS SHOWN

Pregnenolone Acetate (Orally)	*Number of Cases*	*Grade of Therapeutic Response*			
		I	*II*	*III*	*IV*
400 Mg. daily or less	18	.	.	4 (22 per cent)	14
More than 400 mg. daily	26	.	3	10	13
			(50 per cent)		
Total	44	.	3 (7 per cent)	14 (32 per cent)	27 (61 per cent)

of wellbeing or strength, and was usually not directly related to the rheumatic disease.

The value of pregnenolone in maintaining the benefits of cortisone or ACTH was studied. Fig. 2 illustrates the results in a patient who received cortisone for short periods separated by periods without treatment, after which he later received pregnenolone. Note that the benefit always

FIG. 2. An example of failure of pregnenolone acetate to maintain the benefits of cortisone acetate in a patient with rheumatoid arthritis.

occurred when cortisone was injected, but when its use was discontinued, the arthritis always became worse. After the fourth period of cortisone administration, an attempt was made to maintain the improvement by the use of 400 mg. of pregnenolone acetate orally, daily. That this failed is shown by the fact that the clinical manifestations of arthritis intensified to the original level and the sedimentation rate returned to the pre-cortisone level.

In Fig. 3 are shown results following the use of cortisone in a different

FIG. 3. Another example of failure of pregnenolone acetate to maintain the benefits of cortisone in a patient with rheumatoid arthritis.

way. After an initial good response from cortisone, it was injected only two or three times weekly. Slowly the arthritis became worse, then a period of "booster" treatment (daily injections) of cortisone was given and clinical improvement in the arthritis and reduction of sedimentation rate again occurred. However, when pregnenolone acetate was given orally in doses of 400 mg. orally, daily, the arthritis promptly became definitely worse.

FIG. 4. Showing the failure of pregnenolone acetate to supplement the effect of submaintenance doses of cortisone acetate when both steroids were given simultaneously to a patient with rheumatoid arthritis.

In another individual (Fig. 4), cortisone was given orally for a very short period of time. The improvement did not continue after cortisone was stopped. Cortisone then was used parenterally to effect a desired level of improvement, following which an amount of cortisone was employed, which in this case would be submaintenance, and with it pregnenolone acetate was given in dosages of 400 mg. orally, daily. The graph shows that this amount of cortisone was submaintenance and that pregnenolone did not contribute any benefits. The disease became worse to the degree that existed before steroids were used, and again improvement occurred when an adequate amount of cortisone was administered.

These examples (Figs. 2, 3, and 4) illustrate what was observed in many patients to whom pregnenolone was administered after cortisone. That

pregnenolone had no value when given directly following cortisone was thought to be possibly due to the failure of the adrenal glands to convert pregnenolone to one of the adrenal cortical hormones because of the suppressing effect of cortisone on the adrenals. However, the same disappointing results were obtained when pregnenolone was given after ACTH in seven patients; although in one of these seven patients, improvement following ACTH cessation lasted two weeks. Then the clinical condition became gradually worse while pregnenolone was being continued, so that at the end of four weeks the pre-ACTH status again resulted. In all other six patients, the disease promptly became worse after ACTH was stopped, even though pregnenolone acetate was used orally in doses of 400 mg. or more daily.

To further clarify the value or lack of value of pregnenolone in patients with rheumatoid arthritis, a "blindfold" test was made as follows: Tablets of pregnenolone and pregnenolone acetate and tablets of an inert substance to use as a placebo were generously supplied for study. All tablets had the same appearance and it was not known to the patients or the investigators until after the studies were completed which tablets were steroid and which were placebo. Eight patients were given tablets from one supply for four weeks or more and then without their knowledge, tablets from the other supply were used in the same way. Half of the time the patients received placebo and half of the time either pregnenolone or pregnenolone acetate. The number of tablets used was such that when the steroid was administered it was in the dose of 400 mg. a day. Minor improvement occurred in some of these patients but when the key to the testing program was supplied and the score tabulated for pregnenolone and for placebo tablets, no difference in results could be detected.

Summary

These experiences clearly indicate that as used in these investigations in patients with rheumatoid arthritis, practically all the steroids studied were found to be completely devoid of therapeutic value. In one patient, Δ^5, 3 acetoxy-etiocholenic acid seemed beneficial, but studies in more patients will be required before the effects of this steroid can be known. Pregnenolone, pregnenolone acetate, and acetoxypregnenolone were valueless in the majority of patients. The preparations given intramuscularly occasionally caused local irritation. In only 39 per cent of the patients studied was any degree of benefit noted. This was noted more often with larger doses, but for the most part this was only symptomatic improvement and to a minor degree; often the improvement was not directly related to the rheumatic disease, but rather was reported as an improved state of general wellbeing, and a slight increase in strength or endurance. In only three patients (7 per cent) was improvement enough to be graded II in American Rheumatism Association criteria for therapeutic response, and in these three patients the improvement did not continue or could not be repeated. Pregnenolone did not augment the effects of cortisone or ACTH, nor was it effective in maintaining the

benefits effected by cortisone or ACTH after these preparations were discontinued. The lack of dependable, significant benefit from pregnenolone was clearly shown by the blindfold studies using placebos, in which the clinical effects of pregnenolone preparations were not significantly different from those of the placebo.

Discussion

Discussion on this paper follows Chapter 17.

16

The Possible Prolongation of ACTH and Cortisone Effects with Pregnenolone*†

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The beneficial effect of ACTH and cortisone in several diseases is unquestioned. Disadvantages in their use include possible serious undesirable complications. Any procedure which might prolong the beneficial effects of these drugs and decrease the dangers and complications would be highly desirable. Because of the reported favorable influence of pregnenolone in rheumatoid arthritis without any undesirable effects, it seemed logical to attempt to prolong the highly beneficial effects of ACTH or cortisone by pregnenolone. It was our hope to devise a cheap and convenient therapy based on a continuation of this drug starting with ACTH or cortisone and continuing with pregnenolone. After three small series of observations on this problem the question remains unsettled. We are presenting our preliminary observations at this time with indications for further studies.

In the first series, nine patients with undoubted rheumatoid arthritis (grades II and III, stages 3 and 4, according to Steinbrocker's classification) were given 300 mg. of ACTH (Wilson) in nine days. For the first three days they received 20 mg. of ACTH every eight hours followed by two days with 10 mg. every eight hours and finally four days with 5 mg. every eight hours. ACTH was discontinued and pregnenolone or placebos of 200 mg. three times a day (a total of 600 mg.) were given orally for 14 days. The patients were hospitalized for at least a part of the study.

The three women in the series (Table I) had excellent immediate clinical benefit. This included marked relief of pain and stiffness, increased range of joint motion, increased muscular strength and physical activity, marked improvement of appetite, and feeling of well being. Improvement persisted at a maximum level after the dose of ACTH was reduced and throughout the period of pregnenolone administration. In

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TABLE I

FIRST SERIES

DURATION OF RELIEF AFTER ACTH

ACTH (Wilson)		300 mg. in	9 days
PREGNENOLONE		600 mg. ×	14 days
BL♀	12 wks.	BO♂	4 days
HA♀	15 days	FA♂	1 day
KL♀	15 days	KO♂	3 days
		RE♂	1 day
PLACEBO			
BA♂	2 days		
CR♂	3 days		

two instances, symptoms began to reappear within twenty-four hours and the condition became worse so that the pretreatment condition was reached in about one week. The third patient remained greatly improved for an additional 10 weeks. The men noted quite a different experience. All of them began to relapse when the dose of ACTH was reduced. This was so striking that in three cases the dose of ACTH was increased before pregnenolone was started. Even under these circumstances relapses occurred within one to four days after ACTH was stopped and pregnenolone was started. The experience of the two men receiving placebos instead of pregnenolone was identical.

Since the women seemed to have some prolongation of ACTH effects during pregnenolone administration, a second series was planned. It was decided to confine the observation to women, increasing the dose of ACTH to ensure maximum effect when pregnenolone was started. After 20 mg. of ACTH (Wilson) every eight hours for six days, the patients were switched to pregnenolone or a placebo, of 200 mg. three times a day for 21 days. These substances were designated as Compounds A and B without further identification and were so used in the experiment. All patients had excellent immediate clinical benefit from ACTH as noted in the first series.

TABLE II

DURATION OF RELIEF AFTER ACTH

360 mg. in 6 days		
COMP. A	KL♀	4 Wks.
600 mg. × 21 days	LE♀	4 "
	TE♀	6 "
	SH♀	2 "
	BO♀	3 "
COMP. B	BL♀	6 "
600 mg. × 21 days	BO♀	3 "
	KL♀	4 "

Five patients on Compound A (Table II) enjoyed relief from two to six weeks, a mean of four weeks. Three patients on Compound B enjoyed relief from three to six weeks, a mean of slightly more than four weeks. One patient on Compound B is still well 10 weeks after ACTH was stopped; this was the same patient who had benefit lasting 10 weeks after pregnenolone was stopped in the first series.

In the third series six women received cortisone, 900 mg. in six days, 300 mg. the first day, 200 mg. the second and 100 mg. for four subsequent days. After cortisone was discontinued, three women each received Compound A and three each received Compound B for 21 days (Table III).

TABLE III

DURATION OF RELIEF AFTER CORTISONE

Cortisone .....900 mg. in 6 days
Comp. A or B .....600 mg. × 21 days

Comp. A		*Comp. B*	
DE ♀	3 Wks.	CO ♀	5 Wks.
MI ♀	3 "	EK ♀	No Relief
TU ♀	5 "	ST ♀	1 Wk.

Three women receiving Compound A and one woman receiving Compound B noted relief of the same order as was seen with ACTH. Two women on cortisone and Compound B noted no clinical effect from cortisone. It happens that both had rheumatoid arthritis grade III, stage 3 for over 15 years. Their disease was apparently in an inactive stage. Three women receiving cortisone and Compound A had relief from three to five weeks after cortisone was discontinued. One woman had the favorable effect of cortisone continued for five weeks with Compound B. One of the women who noted no benefit during cortisone administration had relief of pain for one week with Compound B.

TABLE IV

DURATION OF RELIEF

	Cases	*Treatment*	*Relief*
	After ACTH		
Pregnen.	3	6 Wks.	16 Wks.
Comp. A	5	15 "	19 "
Comp. B	3	9 "	13 "
	After Cortisone		
Comp. A	3	9 "	11 "
Comp. B	1	3 "	5 "

Data from the three series are shown in Table IV. It is obvious that there is no striking difference between the effects of pregnenolone, Compound A, and Compound B. It is impossible to decide from the evidence

as to whether Compound A or B is pregnenolone and which is the placebo.

Laboratory data in the second and third series showed that the ACTH and cortisone produced marked effects on eosinophils, total white blood count, and on 17-ketosteroid excretion. Both eosinophil and total leukocyte counts were made daily during a three-day control period, the six-day injection period, and for three days after stopping ACTH and cortisone. The data are not complete because of omissions on Sundays and holidays. Inasmuch as counts were all done in the morning and effects of treatment were not detectable until late in the afternoon on the next day, all blood counts were tabulated on the day before they were actually made. The numbers in parentheses indicate the number of observations considered in each instance.

TABLE V
EOSINOPHILS IN R. A.

ACTH (Wilson) .....360 mg. in 6 days
Cortisone .....900 mg. in 6 days

	Control	*ACTH*	*Comp. A*	*Comp. B*
Aver.	125 (19)	8 (44)	160 (10)	162 (7)
		Cortisone		
Aver.	150 (11)	77 (31)	170 (5)	83 (6)

Data on eosinophil counts are shown in Table V in summary form as averages. It will be seen that the average eosinophil count of 125 during the control period fell promptly under ACTH therapy to a very low level and returned immediately to the pretreatment level as soon as ACTH was discontinued. The same change, though not to such a marked degree, was seen with cortisone. No significant difference was recognized between the effects of Compound A or Compound B in prolonging the ACTH effect on eosinophil counts although the count remained depressed on Compound B following cortisone therapy.

TABLE VI
LEUKOCYTES IN R. A.

ACTH (Wilson) ....360 mg. in 6 days—9 cases
Cortisone .....900 mg. in 6 days—6 cases

	Control	*ACTH*	*Comp. A*	*Comp. B*
Aver.	5.4 (12)	9.3 (37)	9.5 (10)	8.3 (6)
		Cortisone		
Aver.	7.2 (8)	8.4 (30)	9.1 (6)	8.1 (9)

Table VI shows a definite rise in total leukocyte count, more marked with ACTH than with cortisone, which was partially sustained during six days of Compounds A or B. There was no significant difference in effect between the two drugs.

Quantitative urine collections were made starting from three to six days before, continuing through, and for six days after all treatment was stopped. Urines were analyzed for 17-ketosteroids in three-day samples and results reported as changes in excretion in milligrams per day from the pretreatment control period. The numbers in parentheses indicate the number of samples considered. It is obvious that there is a marked increase in 17-ketosteroid production during ACTH administration (Table VII),

TABLE VII
17 KS. AFTER ACTH

Days of Experiment	*Mean Change mg./day*	
1–3	+11.9 (6)	
4–6	+15.1 (4)	
	Comp. A	*Comp. B*
7–9	+3.3 (2)	+9.5 (3)
10–12	−0.5 (3)	+1.7 (2)
13–15	−2.1 (2)	−1.2 (3)
16–18	−1.9 (2)	−3.4 (2)

as was expected. This seemed to be sustained at a higher level and for a longer period with Compound B than with Compound A. During cortisone therapy (Table VIII) there was a modest decrease in 17-ketosteroid excretion with a gradual return to pretreatment level with both Compounds A and B.

TABLE VIII
17 KS. AFTER CORTISONE

Days of Experiment	*Mean Change mg./day*	
1–3	−0.2 (4)	
4–6	−0.9 (5)	
	Comp. A	*Comp. B*
7–9	+ 3.2 (2)	+0.2 (3)
10–12	+ 1.3 (2)	−1.3 (1)
13–15	+10.0 (1) ?	−0.9 (2)
16–18	− 3.0 (1)	−1.5 (2)

ACTH (Wilson) was used throughout these observations. This material is said to be about two and one-half times as strong as the ACTH of Armour. That it is effective is indicated by the fact that 26 of the 44 eosinophil counts were zero during the period of ACTH administration, with an average of eight in 44 determinations compared to 77 in 31 determinations during cortisone. The clinical response was prompt and marked in every case. Surprisingly enough, clinical improvement, after ACTH was discontinued, was sustained for longer periods than we had

expected, whether it was followed by Compound A or Compound B. No complications were observed. This experience suggests that it might be desirable to observe a larger series of patients with rheumatoid arthritis treated for short periods with large doses of ACTH. Although these experiments failed to show whether or not pregnenolone prolongs the benefits of ACTH or cortisone, they did indicate certain conditions which must be fulfilled to solve the problem. After ACTH (Wilson) therapy, 300 mg. in six days, female patients with arthritis such as used in this series can expect clinical relief for as long as six weeks after therapy is stopped. To test the influence of pregnenolone or any other substance in prolonging benefit, the test substance will have to be administered at least eight to twelve weeks.

Authors' Note

After these studies were completed and the manuscript written, the senior author (R. M. S.), who did all of the clinical work, learned that Compound A was pregnenolone and Compound B was the placebo.

Discussion

Discussion on this paper follows Chapter 17.

17

Clinical Observations with ACTH and Various Steroids in the Maintenance of Remission in Rheumatoid Arthritis*

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The treatment of rheumatoid arthritis with known methods unfortunately is not curative. It is common knowledge that when a therapeutic agent such as gold, cortisone, or ACTH is discontinued, the disease sooner or later recurs, usually sooner. Much has been written about producing remissions in rheumatoid arthritis with the newer agents of ACTH and cortisone but very little has yet been published regarding the long-term attempts to maintain remission.

ACTH

One hundred sixty-eight patients with rheumatoid arthritis have been treated with ACTH with adequate follow-up. Thirty-five patients with active rheumatoid arthritis have been kept on continuous administration of ACTH for an average of 9.6 months, the longest period being 20 months. This was a selected group of patients who had extremely active disease with minimal joint destruction and who responded well initially to ACTH. Seven patients, or 20 per cent, of this group have maintained complete clinical remission on relatively small doses of ACTH without toxic effects (Grade I except for sedimentation rate). Fourteen patients, or 40 per cent, of this group were able to maintain worthwhile improvement, estimated at more than 50 per cent (Grades II to III). Some of this group experienced mild toxic symptoms and required various adjustments in dosage and all known procedures for the prevention of toxicity. Fourteen patients, or 40 per cent, were classed as unsatisfactory (Grade IV), having maintained less than 25 per cent improvement; in most of this group, edema, acne, round face, fatigue, and tachycardia were constant

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problems. Interestingly enough, most of the unsatisfactory group developed resistance to the drug so that larger and larger doses were required to control the disease. One group of these patients responded dramatically to beef ACTH, indicating that the resistance was to the pig fraction. Others responded neither to beef nor pig ACTH. This study on ACTH was reported in more detail at the Second Clinical ACTH Conference.

Cortisone

One hundred twenty-one patients with rheumatoid arthritis have been treated with cortisone with adequate follow-up. Of this total, 94 received cortisone by the course method, receiving from two to six courses of cortisone. Of this group of 94 patients, only four retained sufficient improvement to be worthwhile, following discontinuance of the medication. Thirty-six patients have received continuous maintenance doses of cortisone (Table I). The average weekly dose has been 375 mg., varying

TABLE I

CORTISONE IN RHEUMATOID ARTHRITIS

No. of patients treated by continuous medication	36
Dosage—Average weekly (200-800 mg.)	375 mg.
Duration—Average (2-8 mos.)	4.4 mos.
No. holding complete remission (Grade I)	2
No. holding worthwhile improvement (Grades II and III)	24
No. failures or unsatisfactory	10
No. with toxic effects; edema, moon-face, etc.	15

from 200 to 800 mg. The average duration of treatment has been 4.4 months; the number of patients with complete remissions is 2; the number with worthwhile remissions is 24; the number of failures is 10; the number showing toxic manifestations, usually in the form of edema or moon-face, was 15. Not all of the toxic manifestations were of sufficient severity to require discontinuance of medication. Several of the patients continuing to show satisfactory remission with the minor manifestations of toxicity are still under treatment.

These figures are not quoted as a comparison between ACTH and cortisone because they are not comparable in selection of patients or in duration of treatment. Rather, they are presented to point out that with both ACTH and cortisone there remains a very sizable group of patients whose disease is not well controlled and who have undesirable physiologic side-effects. We are, therefore, interested and concerned in the search for other antirheumatic substances in an effort to increase the number of patients who can be maintained in remission safely and satisfactorily.

In our search for better methods of maintaining remission, we have screened many different substances during the past two years for antirheumatic activity. Among the substances tested were several 11-desoxy steroids. Because of the fact that we had determined quite early that

cortisone, a steroid, was active orally, and, owing to the difficulties attending the injection method, we decided to use the test steroids orally.

11-Desoxy Steroids

Oral $\Delta^{5,16}$-Pregnadiene-3β-ol-20-one Acetate. It will be noted in Table II that of 11 patients, two showed objective improvement. Three of the 11 patients showed subjective improvement, stating that they felt better.

TABLE II

ORAL $\Delta^{5,16}$-PREGNADIENE-3β-OL-20-ONE ACETATE

No. of patients	11
Dosage	600 mg.
Duration	3-5 weeks
No. showing objective improvement	2
Degree—Grade II, 1; Grade III, 1	
No. showing subjective improvement	3
No. showing drop in sedimentation rate	1

TABLE III

ORAL ALLOPREGNANE-3β, 21-DIOL-20-ONE DIACETATE

No. of patients	13
Dosage daily	400-600 mg.
Duration of treatment	3-12 weeks
No. showing objective improvement	4
Degree—Grade II, 1; Grade III, 3	
No. showing subjective improvement	6
No. showing sedimentation rate drop	2
No. (of 4) showing repeated objective improvement and worse with placebo	3

Oral Allopregnane-3β, 21-diol-20-one Diacetate. Of 13 patients given this steroid, four showed objective improvement; however, only one improved to Grade II, and three to Grade III (Table III). Six expressed subjective improvement. However, when the four patients showing objective improvement were given placebos as a substitute, three of the four showed increase of the disease and improved again on reinstitution of the steroid. The number showing drops in sedimentation rate are reported, not because it is essential to remission, but for the record.

Oral Allopregnane-21-ol-3, 20-dione Acetate. Of the 11 patients studied with this material, two showed objective improvement (Table IV). It will be noted that they both had rheumatoid spondylitis. Three patients showed subjective improvement. Of the two patients showing objective improvement, only one could detect the difference when placebo medication was used.

Oral Pregnenolone (Δ^5-Pregnene-3β-ol-20-one). Of 10 patients studied from three to nine weeks, three showed objective improvement; two patients improved to a Grade II, both of whom, interestingly enough, had rheumatoid spondylitis (Table V). Five expressed subjective improvement. Again, two patients, both with rheumatoid spondylitis, could detect the difference between placebo therapy and steroid therapy, being better with the steroid and worse with the placebo.

TABLE IV

ORAL ALLOPREGNANE-21-OL-3, 20-DIONE ACETATE

No. of patients	11
Dosage	400 mg.
Duration	3-6 weeks
No. showing objective improvement Degree—Grade II, 1; Grade III, 1	2 (both with rheumatoid spondylitis)
No. showing subjective improvement	3
No. showing sedimentation rate drop	0
No. (of 2) showing repeated objective improvement and worse with placebo	1

TABLE V

ORAL Δ^5-PREGNENE-3β-OL-20-ONE ACETATE (PREGNENOLONE ACETATE)

No. of patients	10
Dosage	800 mg.
Duration	3-9 weeks
No. showing objective improvement Degree—Grade II, 2 (with rheumatoid spondylitis); Grade III, 1	3 (2 with rheumatoid spondylitis)
No. showing subjective improvement	5
No. showing sedimentation rate drop	0
No. (of 3) showing repeated objective improvement and worse with placebo	2 (with rheumatoid spondylitis)

Possible Synergistic Effects

We are testing a number of other steroids but as yet have insufficient cases to report. As one looks at these reports on the treatment of active rheumatoid arthritis, one certainly is not impressed that these substances used alone are powerful antirheumatic agents. Yet there is the suggestion in a small percentage of patients that antirheumatic effect is present. It is curious that with allopregnane-21-ol-3, 20-dione acetate and pregnenolone, rheumatoid spondylitis patients appear to be more favorably affected than the peripheral rheumatoid patients. Because of this implied evidence that some antirheumatic activity might be present, we determined to try another method of measuring antirheumatic activity. Patients who were on cortisone maintenance were gradually reduced in dosage, usually to

25 or 50 mg. daily, or to a level at which symptoms began to recur. Then another steroid was added to the routine. The results of one study of this character are illustrated in Table VI. Seventeen patients being continued on cortisone at 25 to 50 mg. daily, all of whom were having symptoms, were given 800 mg. daily of pregnenolone. Of the 17 patients, eight showed objective improvement after the addition of pregnenolone to cortisone therapy. Twelve patients expressed subjective improvement after the addition of pregnenolone. These are as yet short-term cases, and it remains to be seen whether patients can be maintained for long periods on very small doses of cortisone plus a supplementary amount of another steroid which does not produce adverse physiologic side-effects. It will be noted that only three out of ten patients showed any degree of improvement when given pregnenolone alone (Table V). Two of these had rheumatoid spondylitis. Eight of 17 patients showed objective improvement when

TABLE VI

MAINTENANCE OF REMISSION

Oral cortisone (25-50 mg.) + pregnenolone	800 mg.
No. of patients	17
Duration of cortisone before pregnenolone	2-8 weeks
Duration of cortisone + pregnenolone	2-9 weeks
No. showing objective improvement after pregnenolone	8
No. showing subjective improvement after pregnenolone	12
No. showing sedimentation rate drop after pregnenolone	1

pregnenolone was used as supplementary to small doses of cortisone (Table VI). All of these patients had peripheral rheumatoid arthritis. Similar studies are being conducted at the present time with ACTH and several of the other steroids, with similar results already evident in a few cases.

These results are not conclusive, but certainly suggest that in the presence of small amounts of ACTH or cortisone, the antirheumatic effect may be enhanced by the supplementary use of other steroids with a relatively low degree of primary antirheumatic activity. This thought is further borne out by the fact that we have been able also to produce initial remissions combining small doses of cortisone with another steroid, where cortisone alone or the steroid alone would not produce the remission. The number of these cases is small, but again suggests that there may be some synergistic effect with the two substances.

Conclusion

Cortisone and ACTH are powerful antirheumatic agents and will induce remission in a high percentage of patients with active rheumatoid arthritis. The maintenance of long-term remission is more difficult. A definitely worthwhile number of patients can be maintained with long-term administration of ACTH and cortisone but there is also a fair-sized

group of individuals who cannot safely maintain long-term remission with these substances because of failure to control the disease or the development of adverse physiologic side-effects. Because of these facts, a search has been made for other substances possessing antirheumatic activity but with little, if any, adverse physiologic side-effects. Several of the 11-desoxy steroids have been tested. The antirheumatic effect of these substances tested has not been marked, though the evidence may suggest the possibility of an antirheumatic effect, at least in certain individuals. Suggestive evidence is offered, however, that when these steroids are used as supplementary medication with small doses of cortisone or ACTH, in approximately half the individuals studied, there appears to be a synergistic effect producing greater antirheumatic activity than the sum of their individual antirheumatic activity when used alone.

Certainly the search for other antirheumatic substances must go on and, if possible, the specific mechanism of the remission be identified.

Discussion of Chapters 15, 16, and 17

J. Robles Gil: Dr. Stecher, were these true remissions after hormone was stopped? It's rather unusual to have a remission or relief after stopping the treatment.

R. M. Stecher: In the first series, patients receiving ACTH had a relapse as soon as ACTH was stopped. The surprise of the last two series was the long duration of remission of symptoms in the patient who received ACTH. We used Wilson's ACTH, which is said to be two and one-half times as potent as the ACTH of Armour. We believe that this may be so because the effect following its use was very striking. All the patients who received ACTH (Wilson) experienced marked improvement within 24 hours; this improvement increased throughout ACTH treatment. One of the outstanding features of this ACTH (Wilson) treatment was the fact that the patients had flushing of the face. We were much surprised to find that improvement continued for several weeks after ACTH (Wilson) therapy was stopped. There was not enough difference between the effects of the two substances used after ACTH therapy to enable me to distinguish which was pregnenolone and which was the placebo. The effects of the two substances on eosinophil counts and total leukocyte counts were also indistinguishable. Improvement after cortisone was not as great and it did not occur as quickly in the six-day period as after ACTH (Wilson) therapy. The changes in the eosinophil counts and the total leukocyte count were not so great as during ACTH therapy. They were, however, in the same direction and of the same order. I stated that two patients did not respond. They had advanced rheumatoid arthritis of long duration, but the arthritis was in an inactive stage. Patients on cortisone ordinarily do not relapse within a week of stopping therapy so we were not surprised that remissions from cortisone lasted so long. Again, however, we were not able to distinguish any difference between placebo and pregnenolone in maintaining remissions after cortisone withdrawal.

F. Homburger: I should like to add some data to those just presented. We have done a study along the line of the investigation presented by Dr. Stecher in a group of patients with rheumatoid arthritis. We have followed routine courses of ACTH (Armour, Wilson, or Searle) by prolonged courses of pregnenolone. These [cases of rheumatoid arthritis] were all moderate to severe chronic cases but we insisted that there be evidence of activity such as at least one acutely swollen joint, and an accelerated sedimentation rate. Some patients received only ACTH and some additional pregnenolone. There were some instances of remarkably prolonged remissions following ACTH alone. If one included these unusually long remissions in calculating the duration of the average remission induced by ACTH alone, this mean would be 102 days. In view of the fact that such prolonged remissions are unusual they were omitted in calculating an "adjusted average" of the duration of remissions induced by ACTH alone and excluding any case with a remission exceeding 90 days. This "adjusted" duration of remission obtained with ACTH alone in single courses was 36.6 days. When pregnenolone was given immediately following ACTH therapy, the duration of the average remission induced by ACTH was only 21 days. Thus pregnenolone given after ACTH did not prolong the remission induced by ACTH. These findings are summarized in the following three tables.

RESULTS WITH ACTH ALONE

PATIENT			HISTORY		ACTH		REMISSION	
	AGE	SEX	SEVERITY	DURATION YEARS	MG./DAY	DAYS	DEGREE	DURATION DAYS
S.S.	62	F	SEVERE	16	100[A]	12	NONE	
E.R.	30	F	SEVERE	12	100[S]	12	GOOD	90
					100[A]	12	GOOD	500
C.D.	47	M	SEVERE	2 1/2	100[A]	12	GOOD	20
					100[W]	12	GOOD	3
C.E.	47	F	SEVERE	15	200[W]	14	FAIR	6
C.G.	18	F	SEVERE	12	200[W]	12	FAIR	7
R.D	73	M	SEVERE	4	100[A]	12	GOOD	14
J.K.	38	M	MODERATE	2	200[W]	12	GOOD	180
R.S.	7	M	MODERATE	5	50[A]	12	EXCELLENT	410
D.L.	42	F	SEVERE	20	100[A]	12	EXCELLENT	90
					200[W]	14	GOOD	30
E.C.	37	F	MODERATE	12	100[A]	12	EXCELLENT	70

A – ARMOUR
W – WILSON
S – SEARLE
} EXPRESSED IN ARMOUR EQUIVALENTS

RESULTS WITH ACTH AND PREGNENOLONE

PATIENT			HISTORY		ACTH		PREGNENOLONE		REMISSION	
	AGE	SEX	SEVERITY	DURATION YEARS	MG./DAY	DAYS	AFTER ACTH MG. PER DAY	DAYS	DEGREE	DURATION DAYS
M.G.	52	F	MODERATE	1	200[A]	20	800	15	NONE	
B.L.	47	M	MODERATE	1	50[W]	7	[X]800	7	NONE	
					100[W]	14	800	14	QUESTIONABLE	
					—	—	800	70	NONE	
G.L.	26	F	ACUTE, MODERATE	1	100[A]	15	800	44	GOOD	12
E.C.	35	F	MODERATE	12	100[A]	12	800	79	FAIR	[NR]49
A.M.	75	F	MODERATE	7	100[A]	12	800	30	GOOD	14
H.K.	49	F	MILD	3	100[A]	12	800	45	GOOD	21
					100[W]	12	800	45	GOOD	14
E.C.	60	F	MODERATE	10	100[W]	12	800	30	FAIR	14

A – ARMOUR } EXPRESSED IN ARMOUR EQUIVALENTS
W – WILSON }
X – ACTH GIVEN WITH PREGNENOLONE
NR – PREVIOUS REMISSION AFTER ACTH WITHOUT PREGNENOLONE WAS BETTER AND LASTED 10 WEEKS

SUMMARY OF RESULTS

PATIENTS		ACTH		PREGNENOLONE		REMISSION	
NUMBER	COURSES	MG. PER DAY	DAYS	MG. PER DAY	DAYS	DEGREE	DURATION DAYS
7	10	100-200	7-15	500-800	7-75 AVERAGE 37.5	NONE (2) TO GOOD	12-49 AVERAGE 21
10	13	100-200	12	NONE		NONE (1) TO EXCELLENT	6-410 X ADJUSTED AVERAGE 36.6 AVERAGE 102

X – REMISSIONS OVER 90 DAYS ELIMINATED

S. H. Bassett: I should like to ask Dr. Homburger and Dr. Stecher how they withdrew the ACTH. Did they withdraw it abruptly or did they withdraw it gradually, because all this may have an important bearing on the duration of the remission. It has been our impression in using

ACTH that if one withdraws it abruptly, the patient then goes into a severe state of adrenal insufficiency temporarily, and if it is withdrawn gradually, one can maintain the adrenal at a very high functional level. Possibly this maintenance of the adrenal at its higher functional state may have a bearing on the duration of the remission.

F. Homburger: In answer to Dr. Bassett's question, we tapered off the dosages of ACTH by adding at the end of treatment two days of 50 mg. per day and two subsequent days of 25 mg. per day.

R. G. Sprague: In Dr. Freyberg's cases it is noteworthy that when improvement did occur during administration of steroids it was usually mild in degree and subjective in character. I wonder how much credit can be given to the steroids for this improvement. I'd like to ask Dr. Freyberg if he would care to express an opinion whether the improvement was due to the steroids or to other factors.

R. H. Freyberg: That is a very important question. I emphasized in my paper that when improvement occurred, with very few exceptions the improvement was minor. In regard to the nature of the improvement, in most cases when the patients reported improvement they said, "I feel better because I have somewhat less pain, or somewhat less stiffness," or, as many of them said, "the improvement is in my general sense of well-being." They were unable to say that any part of the rheumatic disease was improved; rather they had general improvement that they did not relate to the joints or their function. We report that as *minor* and *subjective* or symptomatic improvement, and that was the improvement in all but three of our cases. So, we, too, raised the question as to whether it is justified to credit steroid preparations with this improvement. In order to answer this question we made control observations in eight patients to whom we administered pregnenolone or pregnenolone acetate orally, but to each of those patients we also gave placebo alternating with the steroid and we did not know when we administered the steroid or the placebo, since it was a "blindfold" study. The patient would be given Preparation I for a period of three weeks or longer and then unknown to him, Preparation II was given in the same way that Preparation I was used. The patients would get 400 mg. of pregnenolone or pregnenolone acetate daily when these preparations were used. After we finished the observations and were told which preparation was pregnenolone and which was placebo we could find *no difference* between the two groups. Some minor improvements sometimes reported were equal in the placebo group and in the pregnenolone group. These results, better than the earlier experiments, indicate that usually the steroid cannot be credited with causing any improvement reported by the patient.

I would like to see experiments done with pregnenolone injected into inflamed joints. Discussion this morning has concerned itself as to how steroids may be effective. We reported last June in San Francisco that if

cortisone were injected directly into inflamed knee joints of patients with rheumatoid arthritis, usually there would be improvement in the joint inflammation as shown by a decrease in joint swelling, articular fluid, and reduction in cells in the joint fluid. We feel that this is further evidence that cortisone has its effect directly on connective tissue. The improvements in inflammations of the eye reported when cortisone is instilled locally into the conjunctival sac or when injected into the inflamed tissues of the eye also indicate that cortisone is effective locally at the site of inflammation in the connective tissue. I would be more convinced of the effectiveness of pregnenolone if I could see evidence of benefit directly on the inflamed or abnormal connective tissue. It must be remembered, however, that pregnenolone may be converted to another substance by the adrenal cortex.

Another way to evaluate therapeutic value of a preparation is to determine whether the patient is willing to pay for the medication after he has used it for awhile supplied to him gratis. This was put to test in the present problem. Our preparations of pregnenolone were supplied to us for a period of time so that we could give them to patients without any cost for doses of 400 to 800 mg. a day. After our investigations were completed and our supply of gratis steroid was exhausted, we needed to tell our patients we did not have any more medication to give them, but that it was available for purchase, it costs so much a tablet, and a prescription for it would be gladly supplied to them. I think it highly significant that the vast majority of them, although they were able to pay, did not get the medication.

A. Segaloff: Many of the compounds which have been mentioned today have rather definite activity in the adrenalectomized animal, which resembles the activity of desoxycorticosterone, particularly in the production of growth in the adrenalectomized animal. However, very little has been said about desoxycorticosterone, yet most of us nonrheumatologists have been reading about desoxycorticosterone, particularly in combination with ascorbic acid. I, for one, would greatly appreciate a few words as to what the present status is of this type of therapy.

Dr. Weiss of our group, using both pregnenolone and placebos, has been following his patients while he was totally unaware of what medication the patients were being given. We have completed a fair period of study in some nine patients now and I have a few slides showing some of the data. Using the American Rheumatism Association classification, the first figure (Fig. 1) shows one type of response that we have seen, i.e. that there was no definite change in anything about the patient during medication with pregnenolone or placebos. Our pregnenolone dosage was 800 mg. per day by mouth. (In the figures, F.I. = functional improvement, T.C. = therapeutic criteria, and S.I. = subjective improvement.) The next figure (Fig. 2) shows another type of response we have seen that has also been mentioned before; it is being shown largely for emphasis, i.e. that some patients do get an apparent objective change, in this case in

FIG. 1. C.S.C. Office Worker 45 years. Rheumatoid Arthritis (156 mos.).

FIG. 2. A.R.B. Office Worker 43 years. Rheumatoid Arthritis (12 mos.).

FIG. 3. D.C.E. Investigator 50 years. Rheumatoid Arthritis (36 mos.).

both subjective improvement and therapeutic criteria, while on placebos. This was maintained while also on pregnenolone and the subjective improvement continued to higher levels on pregnenolone.

The next figure (Fig. 3) shows data with a patient in whom the response bounced up and down. Subjectively this patient improved, got worse, and then improved quite independently of whether the medication was pregnenolone o. placebo. The following figure (Fig. 4) shows a patient, and I might add we have still another one like this, who showed very definite improvement while on pregnenolone and his subjective improvement continued to increase although the medication was changed to placebo. However, his functional impairment and the therapeutic criteria remained the same on the placebo. More patients show subjective improvement while getting pregnenolone as opposed to the placebo.

FIG. 4. C.J.A. Barber 43 years. Rheumatoid Arthritis (12 mos.).

The improvements are far less striking than, as has been mentioned before, those seen with either cortisone or ACTH.

G. Djerassi: As a chemist I would like to make a comment on the chemical nomenclature employed. For instance, both in the morning, and now in the evening session, 21-acetoxypregnenolone is considered a pregnenolone derivative. One should not consider this a derivative any more than one would classify Compound S (11-desoxycortisone) as a cortisone derivative. Their biologic properties differ very appreciably: 21-acetoxypregnenolone has a definite desoxycorticosterone-like effect in adrenalectomized rats, while pregnenolone has none or a very dubious one.

My second point is in connection with Dr. Stecher's paper in which he referred to pregnenolone and a placebo as Compounds A and B respectively. This is perfectly all right for differentiation purposes in a clinical investigation but it is extremely confusing in a lecture. Compounds A and B were isolated by Reichstein and by Kendall and they refer to different corticosteroids isolated from adrenals. Since we are also

talking about Compounds L, P, and S in this conference, the use of letters as trivial indices appears very unsatisfactory and should be changed to numbers or perhaps Greek letters.

A. Zaffaroni: I was impressed this morning by the results reported by Dr. Freeman on the effect of pregnenolone in patients with rheumatoid arthritis. According to the data presented there was a good correlation between the age of the patients, the stage and severity of the disease, and the response to pregnenolone therapy. I wonder if similar analysis of the other clinical data reported, e.g. Dr. Freyberg's, might not be worthwhile in helping us to understand varying degrees of responsiveness to pregnenolone.

W. Bauer: The discussion today is reminiscent of the pre-ACTH-cortisone days. Then, as now, our task was that of determining the antirheumatic effect of newly introduced drugs. Then, as today, many agents were claimed to have, if not specificity, at least a definite effect on rheumatoid arthritis. During the pre-ACTH-cortisone period the initial reports on most antirheumatic drugs were strikingly similar, so much so that the therapeutic results were frequently referred to as "the inevitable 75 per cent improvement." The early enthusiasm for each drug was sooner or later dampened by the publication of critical, well-controlled studies with essentially negative results.

The sponsors of this symposium and those of us in attendance who are interested in rheumatic diseases have a common interest, namely, search for a steroid possessing high antirheumatic activity but devoid of adverse effects. In attempting to achieve this common goal we clinical investigators must avoid the known pitfalls of the past; otherwise we shall mislead again our colleagues in chemistry and pharmacology, our patients, and ourselves. These pitfalls can be avoided by rigid adherence to the methods of procedure mentioned this morning and the use of control series when reporting therapeutic results.

I agree with Dr. Zaffaroni that breaking down our data, as did Dr. Freeman, may prove helpful; however, the results should always be compared with those of a control series. We have reported such data on 250 unselected patients with rheumatoid arthritis observed for an average period of 10 years. The most striking factor affecting prognosis was duration of the disease. Approximately 75 per cent of patients with arthritis of one year's duration or less showed some improvement, whereas only 44 per cent of those with disease of one or more years' duration improved. Patients 39 years of age or younger showed a greater degree of improvement than those 40 or over, 62 per cent as compared with 42 per cent. The difference in outcome in the two sexes was not significant, although the males did slightly better, 58 per cent as compared with 50 per cent.

Other interesting facts concerning the clinical course of rheumatoid arthritis noted in this same group of patients under observation for 10 years were: Over 50 per cent evidenced a definite degree of improvement

when last seen; 15 per cent were considered in remission, and 9.2 per cent were judged five-year "cures."

I cannot agree with Dr. Holbrook that one can get an excellent clinical remission or a complete clinical remission with ACTH and cortisone without some fall in sedimentation rate. If he means a partial or incomplete remission, that is different. In the latter, the sedimentation rate and serum proteins determined by means of electrophoresis may not revert toward normal; however, even these instances are exceptional.

One thing we have all learned since the advent of ACTH and cortisone, and more recently desoxycorticosterone and other steroids, is the value of placebos in evaluating antirheumatic activity. This was clearly demonstrated when testing desoxycorticosterone. The first patient treated with this steroid exhibited an excellent response as judged on the basis of pain and functional improvement; however, the same degree of improvement was observed when placebo therapy was administered.

The improvement noted in a single joint following the intra-articular injection of cortisone is difficult to interpret. Dr. Freyberg suggests that it represents a direct effect of cortisone on the inflamed synovial tissues. This may be true; however, I am sure he will admit the possibility of other explanations because of the concomitant improvement in other joints, at times accompanied by a fall in the sedimentation rate. The latter findings indicate rapid absorption of intra-articular cortisone.

T. H. McGavack: Following Dr. Bauer's comments regarding criteria, I am again wondering if it is logical to "screen" one drug by using a second one as an index of reference and on that basis decide whether or not the tested substance has certain definite values. For instance, we have found in using cortisone in a selected group of rheumatoid arthritics that there were no changes in the blood potassium level but if insulin were given for several days with the steroid, then subsequently administered cortisone produced changes in the potassium values after insulin was stopped.

W. P. Holbrook: I think it is very important to define what one means by remission, as it may be of any degree. I was speaking of complete remissions. Such a patient has no symptoms, signs, pain, or disability, and works or goes to school normally. There are five of our seven patients on ACTH at this moment who still have high sedimentation rates and have total absence of any symptoms or signs of the disease. Now, if you refer to a remission as a permanent condition, I'll admit readily that these patients do not have a remission, because they have been stopped periodically to see whether the medication was actually maintaining the remission. In each instance the disease recurred rather quickly. When returned to the medication, the patients again were without clinical symptoms or signs of disease. Now that is what I mean by complete remission. I am sure the disease is not cured. I am sure the patient is not in a permanent state of remission. As far as I can tell by examining him or by watching him work, he is in a complete remission but continues to show an elevated sedimentation rate.

W. Bauer: I have seen this in a natural remission. In these patients there have been no subjective or objective evidence of rheumatoid arthritis. To date I have not seen an excellent ACTH- or cortisone-induced remission without a fall in the sedimentation rate.

F. Homburger: We had one case with Dr. Holbrook's type of response, a seven-year-old patient with Still's disease. This patient had a very high β globulin during the ACTH treatment and a high sedimentation rate for several months which did return to normal. During all this time he was in complete clinical remission. Conversely, we had two patients who did not respond to ACTH but had a return of the sedimentation rates to a completely normal level without any change in their severe rheumatoid arthritis.

R. H. Freyberg: I am sure all who have studied rheumatoid arthritis and used various substances as therapeutic agents have seen exceptions to usual findings. I, too, have seen some patients in whom the change in erythrocyte sedimentation rate is far less than usual and out of proportion to the clinical changes. I do not recall any instances in which the sedimentation rate did not decrease to some significant degree during the course of clinical improvement effected by cortisone or ACTH. But, I wish to emphasize that sedimentation rate measurement is a valuable part of our observations to help determine the value of test drugs. The majority of patients who get significant improvement with ACTH or cortisone have a fall of sedimentation rate but not necessarily to normal. I think that in contrast to these quite consistent reductions in cortisone- and ACTH-treated patients, the failure of reduction in sedimentation rate in all but 3 of 44 patients treated with pregnenolone and other test steroids has definite meaning.

A. R. Abarbanel: In pregnancy, the sedimentation rate begins to increase quite rapidly and yet strangely enough arthritis may be improved. Is this a special condition or is this just a condition where the general rule regarding the sedimentation rate does not apply?

F. Homburger: In our six cases of lymphosarcoma, neurosarcoma, and other miscellaneous tumors treated with ACTH, all had high sedimentation rates. Although all ultimately died, the sedimentation rate in all cases became normal when ACTH was given.

H. Selye: I wonder if Dr. Holbrook would care to comment on the high incidence of unpleasant side-effects after ACTH and cortisone in his series. I think it was a little higher than usually reported. Particularly I would like to know if the sodium intake was controlled.

W. P. Holbrook: Yes, Dr. Selye, I think the difference rests in the length of time of administration of hormone. Remember, with ACTH these patients averaged 9.6 months, some going up to nearly two years, of con-

tinuous administration. All presently known methods of preventing such adverse physiological effects, including sodium restriction, potassium chloride administration, thyroid administration, as well as a variety of other methods, were used in an attempt to prevent and control these symptoms. Only by using these precautions were we able to continue treatment on many of these patients who were maintaining worthwhile improvement. I think that if we will count our cases honestly, including those with minor changes, a similar incidence will be found by other workers with comparable cases and length of administration. I hope better methods will be found for preventing these side-effects.

K. Dobriner: I had hoped that this conference would clarify some questions of great medical importance. I have no axe to grind for pregnenolone, but I cannot understand the differences in results shown by the various investigators. I wish for the clarification of my mind that there would be some discussion of these differences. Dr. Holbrook has 10 cases treated with pregnenolone. There were two objective and five subjective improvements. Dr. Freyberg saw similar subjective improvements in 11 of 20 cases. Dr. Freeman and Dr. Davison saw striking improvement. There is a problem here. I would like to have the clinical investigators get down to brass tacks, and at least let me leave Mexico knowing whether certain compounds are of value or are of no value in therapy.

J. W. Conn: I have been in the same position today in which Dr. Dobriner finds himself. I think the answer to Dr. Dobriner's question lies in the fact that we don't have the basic knowledge with which to evaluate arthritis, or the therapy of arthritis. When Dr. Bauer and Dr. Holbrook have difficulty in agreeing on what constitutes a remission on either cortisone or ACTH, how are we going to compare with those materials a substance like pregnenolone? If it has any antirheumatic activity, it is much less than that exerted by cortisone or ACTH. In terms of metabolic indices, Dr. Holbrook has pointed out, and I think with justification, that there is no single effect of cortisone or ACTH that parallels the remission in every case. It seems to me that prerequisite to resolution of these clinical problems is the establishment of a handle, so to speak, a numerical index by which we can evaluate antirheumatic effects. Screening procedures on a clinical basis lose their meaning in a situation where placebo medication has indicated the importance of the patient's reactivity to any kind of therapy. Prerequisite to any screening of many substances that may have less antirheumatic activity than cortisone or ACTH, we must have some kind of antirheumatic index which can be measured in terms of chemistry or some other objective measurement. Difficult as the problem is, such an index must be sought and found before we can assign, with confidence, lesser degrees of antirheumatic activity to given compounds.

R. H. Freyberg: I emphasized that I wanted to credit the individual case with any change that could be called improvement, no matter how minor.

In so doing, the tabulation resulted in the figure of 50 per cent in patients who had more than 400 mg. of the drug, and in 22 per cent of those who had less. However, when one eliminates the slight lessening of pain or slight lessening of stiffness or increase in strength or other minor evidences that might have been reported as subjective improvement, one has practically nothing left out of our series of cases. These results conform, therefore, with those of Dr. Bauer and others who have said more boldly that they have seen no beneficial effects.

I think there is no discrepancy in most of the reports that were given. I do notice the discrepancy in one or two reports and I, just as anybody else, would like to know why. I had hoped to learn when I came down here why these differences exist in previous reports and I still hope that I shall. Dr. Conn emphasized the great need for uniform and dependable measures of evaluation but they are very difficult to attain and he did not tell us how. I hope that everybody else here will tell us what he can to contribute toward that dependable measure of change in rheumatoid arthritis. I can truthfully say I have never had such difficulty as I have had in studying patients with rheumatoid arthritis. That is why I emphasize that it makes a difference if one compares a test preparation to a substance that has a major antirheumatic effect, such as cortisone or ACTH, or whether one compares it with an analgesic such as aspirin, physical therapy, or the like. It is relatively easy in a disease such as diabetes mellitus where one can measure glucose in the urine or in the blood, or when one can measure phosphorus, sodium, potassium, and other elements, for then one has a dependable quantitative index as to changes that relate to disease. But we do not have such measures for rheumatoid arthritis and that is the dilemma. A committee of the American Rheumatism Association has been working hard for three years to develop a set of criteria that are currently the best means for uniform evaluation of therapeutic results. These have been accepted for clinical trial. There may be important shortcomings in these criteria but they are standards that we can work with and I think if the results of studies are expressed in terms of such criteria, there will be less discrepancy.

R. T. Smith: I think there are a number of problems encountered in the treatment of rheumatic disease that might be lost sight of from time to time. Prior to a year and a half ago, treatment of rheumatoid arthritis was a long-term affair. We had some good methods and we achieved some good results whether we used drugs or didn't use drugs, depending on the city in which we were. Then suddenly we developed miracle drugs which seemed to cure people overnight but let them relapse as soon as the drugs were stopped. Then we had a new impetus and used all the steroids we could get our hands on in an attempt to produce the same miraculous results. I think almost anyone interested in rheumatoid arthritis, in particular, will admit that he will be well satisfied if he can find a form of treatment which takes a little longer than a day or week or two and produces as good results or better than we have obtained in the past. I don't

think it is fair, in view of most of our experiences, to expect to pick out of a hat a miracle drug that will do what ACTH or cortisone will do. Instead, we should be willing to consider some longer-term forms of treatment; not just for a week or two but much longer. When we use gold, it requires weeks and months to accomplish good results. Patients have been satisfied and have gotten better. Are we taking the wrong attitude? Every new drug that is offered for trial is compared with these miracle drugs. I think possibly we are making a mistake. I think in many instances we have taken a shot at a certain drug and dropped it after an inadequate trial. Apparently, in some of the results we have seen here today, there have been some drugs that have been used in the past and considered of no value but now we see some people are benefited by them. For instance, testosterone does have some benefit in the treatment of rheumatoid arthritis when used for long-term treatment. It is necessary to change the sex of a female to a certain extent in order to achieve benefits, but benefit can often be achieved. Treatment with testosterone is not for a week or two but a month or more. So, possibly our aims are set a little too high and we should be willing to try new compounds for a longer period of time, just as we are now planning to use ACTH and cortisone for longer periods to prolong the miracles which we haven't been able to prolong before. In other words, why not use all new steroid compounds for longer periods and judge them on their own merits rather than compare them over short periods with unusual and temporarily beneficial miracle compounds?

18

Metabolic and Clinical Studies with Δ^5-Pregnenolone* and Its Esters

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Medical opinion appears to be somewhat divided regarding the use of pregnenolone* in rheumatoid arthritis and some of the other so-called collagen diseases. Table I summarizes the principal data in the literature concerning the administration of pregnenolone in rheumatoid arthritis. Furthermore, the pharmacological activity of pregnenolone seems to be little understood. In conjunction with the management of several diseases, particularly rheumatoid and osteoarthritis, occasion has arisen to observe the effects of the drug clinically in more than 100 subjects. The results have been sufficiently encouraging to warrant a concomitant investigation of some of its metabolic effects. Three main approaches will be made to the subject: (I) Body balances for several metabolites, including nitrogen, sodium, chloride, and potassium, as influenced by pregnenolone; (II) A summary of clinical findings and results in scleroderma, psoriasis, Addison's disease, various types of arthritis and lupus erythematosus and in several other diseases; (III) A discussion of the action of the drug and some comparisons between it and ACTH and cortisone as derived from both personal experience and a review of the literature.

The Metabolic Effects of Pregnenolone

A. METHODS AND MATERIALS

Seventeen individuals, 14 women and 3 men, ranging in age from 11 to 65 years have served as the subjects of these observations. Most of the methods have been previously described.[19] However, several things should be emphasized. While specific dietary regimens were prescribed, in no instance was the diet forced, except as regards insistence upon a rather

* Pregnenolone as used throughout this study refers to Δ^5-Pregnene-3β ol-20-one. Generous supplies of preparations of this steroid have been made available through the courtesy of Dr. E. L. Henderson of Schering Corp. and Dr. I. V. Sollins of the Chemical Specialties Co., whose courtesy and interest is herewith gratefully acknowledged.

TABLE I

REPORTS OF RESULTS OF TREATMENT OF RHEUMATOID ARTHRITIS WITH PREGNENOLONE

Investigator	No. Cases	Daily Dose (mg.) Range	Daily Dose (mg.) Aver.	Daily Dose (mg.) Route*	Duration of Treatment (days)	Results—Relief Marked	Results—Relief Moderate	Results—Relief None or Slight	Remarks
Guest et al. (1) †	19	100–300	150	I.M.	7–51	1	1	17	
Polley & Mason (2)	1	100–300	...	I.M.	10	0	0	1	
Smith (3)	45	200–600	...	O.	14–56	0	12	33	
	6	200	...	I.M.	14	0	0	6	
	40	200	...	O.	42	7		33	
	20	200	...	O.	42	0	0	20	
	..	500	...	.	28	..	..	..	
Copeman et al. (4)	8	100–300	...	.	10	0	0	8	
Cohen et al. (5)	20	300	...	I.M.	1–131	15	0	5	
Cohen et al. (6) (7)	31	300	...	I.M.	30–120	29	0	2	Other steroids also used
Kling (8)	40	100	...	I.M.	7–28	16		24	
Davison & Koets (9)	12	50–300	...	I.M.	14–75	9		3	
Davison et al. (10)	14	200–300	...	I.M.	21–56	11		3	
Gil and Mont (11)	30	100	...	I.M.	7	23		7	
	..	300	...	O.	30	..	..	..	
Strazza (12)	22	350–400	...	I.M.	?	16	4	2	
	..	300	...	I.M.		..	..	..	
Ehrlich (13)	4	500	...	O.	28	2		2	
Rubin (14)	10	300–600	...	O.	10–42	5		5	
Freeman & Pincus (15)	64	500	...	O.	14–210	24	26	14	
Geschickter (16)	10	100	...	I.M.	7–21	3	4	3	
	..	300	...	O.					
Bastenie et al. (17)	3	50–100	...	I.M.	5–20	3		0	
Ishmael et al. (18)	123	100	...	O.	?	61		62	Adjuvant to other steroids
Total	522	...	...	...	..	88 (137)	47	250	
						272 (52.2%)			

* I.M., intramuscular; O., oral.

† Bibliography references.

definite intake of salt. Each day, an aliquot of every food eaten was analyzed for its content of nitrogen, sodium, chloride, and potassium and the resultant figures were recorded as "intake." The urine was analyzed quantitatively daily throughout the control, test, and recovery periods for its total content of nitrogen, sodium, chloride and potassium. In calculating "balances" from the data obtained as above indicated, fecal nitrogen was placed at 1.0 gm. daily, in line with the suggestion of Albright and his associates[20] (patients with diarrhea were not included in the study). The sodium and chloride of the perspiration were calculated as a percentage of the 24 hour "insensible weight loss," as determined by subtracting the morning weight before excretion of urine or stool and before breakfast from the weight at bedtime immediately after voiding. In this determination, proper adjustment is made for any excreta during the night or any water or other fluid drunk. From the difference in night and morning weights, the total insensible weight loss for the 24 hours is determined and sweat estimated. Sodium of the sweat is calculated as 65 milliequivalents per kilogram, and chloride as 50 milliequivalents per kilogram.

In addition to the above determinations, there were estimated quantitatively daily in the urine creatinine, uric acid, and 17-ketosteroids. In the serum, periodic determinations of total proteins, nonprotein nitrogen, creatinine, cholesterol, sodium, potassium and chloride were conducted.

In the present studies three diets were employed, which *as used by the subjects* were constituted as follows:

I. The regular ward diet which contained 235 gms. of carbohydrate, from 43 to 86 gms. of protein and about 80 gms. of fat to yield 2000 calories per day, more or less. Salt was allowed ad libitum and varied in amounts from day to day and from individual to individual from 83 to 147 milliequivalents (the equivalent of from 4.8 to 8.5 gms. of salt). In fact the extremes were actually reached by one of the three individuals using this diet. Chloride intake varied from 86 to 154 milliequivalents daily (the equivalent of from approximately 5.0 to 9.0 gms. of salt). Potassium intake daily varied from 9.0 to 70 milliequivalents (the equivalent of from 0.7 to 5.0 gms. of potassium chloride).

II. A prepared baby food diet, which contained approximately 250 gms. of carbohydrate, about 90 gms. of fat and from 35 to 95 gms. of protein and was designed to yield approximately 2200 calories daily. The sodium intake varied from 87 to 165 milliequivalents; that for chloride from 83 to 172 milliequivalents; and for potassium from 22 to 62 milliequivalents.

III. A liquid diet with salt restricted except in the case of one subject who received this diet plus an addition of 6.0 gms. (103.5 milliequivalents) of salt. As consumed, the diet contained from 180 to 300 gms. of carbohydrate, 45 to 65 gms. of protein and 65 to 85 gms. of fat. The salt intake actually varied from 22 to 42 milliequivalents daily (the equivalent of from 1.2 to 2.4 gms. of salt).

B. Results

I. Variations in Serum Protein, Cholesterol, Nonprotein Nitrogen, Creatinine, Sodium, Chloride and Potassium. While variations in the above named constituents of the serum were observed from subject to subject and from period to period in any given subject, none of these could be related to the particular dietary regimen or to the administration of pregnenolone.

II. The Excretion of Creatinine and Uric Acid. Considerable fluctuation was observed in the values for creatinine, less so for uric acid, but these changes in no way reflected the action of the steroid compound. A transient depression of the eosinophil count was observed in a majority of the subjects.

Table II

The Excretion of Urinary 17-Ketosteroids in Subjects Receiving Pregnenolone with and without Restriction of Salt

Subject	Period (Averaged daily excretion in mg.) "Before" (Control)	"During" (Pregnenolone)	Averaged Daily Difference
A.—Salt Not Restricted			
R.G.	2.8	5.3	+2.5
B.J.	11.3	13.9	+2.6
H.S.	4.2	7.2	+3.0
I.T.	16.4	26.9	+13.5
S.L.	2.5	3.5	+1.0
A.L.	1.8	2.9	+1.1
F.R.	3.8	4.4	+0.6
B.—Salt Restricted			
A.B.	7.4	7.1	−0.3
S.Q.	8.3	6.1	−2.2
R.R.	6.5	6.1	−0.4
J.S.	12.5	10.7	−1.8

III. The Excretion of 17-Ketosteroids. The behavior of urinary 17-ketosteroids was interesting in relation to two factors; the dosage of pregnenolone employed and the use of a low salt diet (Diet III). In Table II we have listed the averaged excretion in milligrams per day of 11 subjects taking pregnenolone for a three-day period. The column marked "before" is the average of the two control days and the column designated "during" is the average of the three days of administration of the drug together with the first day of the "recovery" period. It will be noted that the seven subjects whose salt was not restricted developed an increase in 17-ketosteroid excretion under the influence of pregnenolone while the four from whom salt was withheld showed a decrease in the urinary 17-ketosteroids.

The increases observed in the seven subjects with an adequate diet and intake of salt are not large and represent a trend rather than a set of statistically significant figures. It is suggested that if the dose of pregnenolone is sufficient, there is a tendency for the excretion of 17-ketosteroids to be increased.

FIG. 1. Balances for ten subjects, three of whom were given diet I, three diet II and four diet III. The negative balances for sodium and chloride reflect the influence of diet III. Note the rapid drop in the positive nitrogen balance and tendency by the third day for an increase in the excretion of sodium and potassium.

When the salt intake is even moderately reduced—in the present experiments to an average of 1.8 gms. daily (0.7 gm. of sodium or 30.4 milliequivalents), this rise in 17-ketosteroid excretion not only does not occur but there is a slight diminution in the amount found in the urine (Table II).

The increase in the excretion of 17-ketosteroids also occurred in the subjects who received pregnenolone and a whole adequate diet (Diet I

or II) for test periods of 11 days. However, this rise appeared to be a transient one as in each instance it had returned to the pretreatment level within 5 days after prenenolone therapy was begun.

IV. The Balance for Nitrogen (Figs. 1 and 2). Three of the 11 subjects on the 3-day test period with pregnenolone and three of the five

FIG. 2. Balances for nitrogen, potassium, sodium and chloride in five subjects to whom pregnenolone in aqueous suspension was administered for 11 days. There is a slight increase in the retention of nitrogen on about the fifth day with a decreasing ability to retain nitrogen from that day onward. A sharp rise in retained nitrogen appears following the cessation of therapy. The concomitantly observed behavior of electrolytes is erratic and apparently assumes no significant trend.

subjects who took the steroid for 11 days were in positive nitrogen balance at the beginning of the test period. The others were "in balance." Therefore, the positive balances seen in the composite graphs (Figs. 1 and 2) reflect these values. Such balances did not seem to influence the general trend of the action of pregnenolone which was to favor the excretion of nitrogen. Fig. 3 is a typical illustration of such an effect. However, there

was a transient increase in the retention of nitrogen beginning on the first day in five of the 11 subjects to whom it was administered for three days (Figs. 4 and 5) and in one of the five who received it for 11 days (Fig. 6). In five of the six subjects in whom this occurred, the nitrogen stored could be fully accounted for by an increased ingestion of protein.

FIG. 3. A.B., a 40-year-old woman with malignant exophthalmos showed a negative nitrogen balance during the last two days of a three-day period of administration of pregnenolone, 400 mg. daily. Intake of salt approximately 1 gm. daily. Under these conditions pregnenolone appears to have aided in the conservation of salt and chloride and furthered the excretion of potassium.

This increased intake is a reflection of the increased appetite which frequently accompanies the administration of the drug, particularly in patients with arthritis whose pain is relieved. Sometimes this relief of pain, improvement in well being and increased appetite appear later. For instance in four of the five subjects in whom balance studies were carried

out for the longer period (Fig. 2) nitrogen retention was most marked about the fourth to sixth days, which at the same levels of intake for the rest of the experimental period became less positive and in some instances negative. In Fig. 6 is graphically depicted a rather characteristic nitrogen balance curve of an individual illustrating this point. Note also the early transient rise in urinary 17-ketosteroid values.

FIG. 4. R.G., a 15-year-old girl, completely recovered from an acute attack of rheumatic fever increased her nitrogen intake from the first day of pregnenolone therapy by an average of 2.0 gms. daily. Note the early increase and later decrease in the amount of nitrogen retained. Sodium, chloride and potassium appear to follow these changes.

From a study of the larger doses of pregnenolone given over an 11 day period, three things seem clear: (1) In some cases there is early an increase in protein intake, associated with the retention of a portion of the extra protein; (2) an increasing loss of protein despite the main-

tenance of protein intake and (3) a marked retention of protein in the "recovery period" (Fig. 2).

The early period of gradually increasing protein intake appears to be due to an improvement in appetite and in general well being of the subject. Only a portion of this nitrogen is retained but this is sufficient

FIG. 5. A.L. Scleroderma and inactive rheumatoid arthritis of many years' duration. Appetite markedly improved by pregnenolone with an average increase in intake of 2.0 gms. of nitrogen daily. Note early rise and later decrease in amount retained. There is no accompanying change in sodium or chloride.

to produce for the first three or four days a further increase in the positive balance present. After this, there is an increasing loss of nitrogen for as long as the steroid has been administered while the balances were studied and for 24 hours thereafter (Figs. 1 and 2). Finally, after stopping the drug, there is a return of the subject to a very positive nitrogen balance, more marked than that observed in the control period (Fig. 2). In the five

cases included in the 18 day study, during 11 of which the subjects were given pregnenolone, the composite graph (Fig. 2) shows that an average of 2.4 gms. of nitrogen were being retained daily by each subject during the control or pretreatment period; that this decreased during the first

FIG. 6. R.M., a 49-year-old woman with grade IV lesions of rheumatoid arthritis, without active lesions at the time of the pregnenolone balance study. The average daily increase in nitrogen intake was 3.5 gms. Note early increase (?) in the excretion of 17-ketosteroids, the definite drop and late tendency to return toward normal.

four days of treatment to an average of 1.7 gms., rose to an average high of 3.1 gms. on the following day in conjunction with a much improved intake of protein and maintained an average of 2.5 gms. per day for four days, after which it dropped to an average of 1.1 gms. for the last three days of drug administration with an actual negative balance appearing

during the first day of "recovery." In sharp contrast is the marked retention of nitrogen observed in each of the five patients on the "11 day run" during the last three days of the recovery period (Fig. 2), the average daily retention per subject being 5.0 gms.

FIG. 7. B.J., a 32-year-old pregnant woman, whose average intake of nitrogen prior to the administration of pregnenolone was 11.6 gms. daily and increased on the second and third days of pregnenolone administration and the first day of recovery by 3.0, 4.4 and 8.5 gms., respectively.

In one subject only was there a continuing increasingly positive balance for nitrogen. This was a 32-year-old woman five months' pregnant with a chronic relatively inactive lupus erythematosus with skin lesions confined to the face. In this individual the balance for nitrogen became and remained strongly positive throughout the five-day period of observa-

tion after pregnenolone was begun (Fig. 7). Did her pregnancy play a part in this type of reaction?

In sharp contrast is the failure of pregnenolone to alter the negative nitrogen balance observed in one patient with Addison's disease who was rapidly losing weight at the time the administration of pregnenolone was begun (Fig. 8). Here it will be observed that a further excretion of nitrogen did not take place, nor was the cessation of drug therapy followed by a retention of nitrogen-containing material.

V. The Balances for Sodium, Potassium and Chloride. Relatively wide fluctuations were observed in the excretion of electrolytes under the influence of pregnenolone. During the first three days, their excretion roughly paralleled the increased excretion of nitrogen (Figs. 1 and 9), except in the group who had a low salt intake, which will be considered

FIG. 8. L.R.W., a 38-year-old woman with Addison's disease of 5 years' duration whose appetite was poor and who was losing weight at the time of the balance study.

separately. During the last four to five days of the longer period of administration, the increasing negative balance for nitrogen was associated with a slight but definite tendency for the retention of these three electrolytes (Fig. 9). This oppositely directed behavior continued into and through the phase of recovery.

A very striking difference from the above was apparent when the amount of sodium and chloride ingested was limited to about 30 milliequivalents daily (Fig. 10). In each of four subjects thus treated, pregnenolone administration was associated with a decrease in the negative balance, this effect being more striking for sodium than for chloride.

Clinical Observations

The pregnenolone preparations and routes of administration used in treating patients have included the intramuscular injection of aqueous suspensions of pregnenolone, pregnenolone acetate, and of 17α-hydroxypregnenolone and the oral use of pregnenolone acetate, and pregnenolone

betaine hydrochloride as tablet triturates. Effort has been made to evaluate both subjective and objective responses in keeping with criteria previously outlined.[21] The results may be properly considered in three categories corresponding to the diagnosis: (1) collagen diseases—93 cases; (2) endocrine disturbances—15 cases; and (3) miscellaneous conditions—7 cases. Some of the data are summarized in Table III. Clinical and laboratory data were collected for all subjects in kind and manner as described in a previous communication.[21]

FIG. 9. Averaged data for sodium and chloride balances in the same cases, the nitrogen and potassium balances of which are shown in Fig. 2.

General Course under Therapy

The reaction to the various preparations employed, irrespective of the route of administration, was a qualitatively similar one. In a majority of subjects the response to pregnenolone acetate intramuscularly given was equal qualitatively and quantitatively to a similar amount of the same ma-

terial per os in the form of a tablet triturate. However, in approximately ten per cent of the subjects, the intramuscular route afforded satisfactory relief while the oral administration of the material was relatively ineffec-

FIG. 10. Influence of variations in salt intake upon the action of pregnenolone. With the salt moderately restricted to 1.8 gms. (cross hatched columns), there is apparently a tendency to retain sodium and possibly chloride.

tive. When allowance was made for differences in molecular weight, the other preparations proved approximately as active as the aqueous suspension of pregnenolone acetate, by injection, which we have accepted as a

Table III

Clinical Application of Pregnenolone

Disease and Sub Group*	No. of Cases	Age Range (Yrs.)	Sex M	Sex F	Severity I	Severity II	Severity III	Severity IV	Dosage Daily Range (mg.)	Dosage Total Aver. (Gms.)	Duration of ℞ (Days) Range	Duration of ℞ (Days) Aver.	Result Moderate to Marked Improvement	Result None to Slight Improvement
ARTHRITIS														
A—Rheumatoid														
I	4	38–56	1	3	1	2	1	0	400–1500	52.4	8–169	86	3	1
II	15	42–64	6	9	1	6	6	2	100–600	20.9	29–30	29	8	7
III	15	43–80	8	7	0	6	8	1	200–1000	17.8	12–97	40	6	9
IV	6	27–65	1	5	0	1	2	3	300–600	30.8	22–123	72	3	3
Total	40	.....	16	24	2	15	17	6	.....	...	.....	..	20	20
B—Osteo-Arthritis														
I	2	56–63	0	2	0	2	0	0	400–600	20.2	8–89	49	1	1
II	12	52–75	2	10	0	6	4	2	60–400	8.5	16–120	36	7	5
III	14	51–86	3	11	0	9	3	2	100–400	19.6	17–132	55	8	6
IV	3	63–75	1	2	0	2	1	0	200–600	19.2	8–60	39	0	3
Total	31	.....	6	25	0	19	8	4	.....	...	.....	..	16	15
C—Gout	3	50–54	3	0	2	1	0	0	90–300	4.0	8–60	33	1	2
D—Allergic Hydrarthrosis	1	38	1	0	1	0	0	0	400–600	14.6	25	28	0	1
E—Acute Arthritis	1	46	0	1	0	0	1	0	50–200	2.25	27	27	1	0
Total Arthritis	76	.....	26	50	5	35	26	10	.....	...	.....	..	38	38

LUPUS ERYTHEMATOSUS														
A—Acute	3	24–40	1	2	0	0	0	3	400–600	3.6	2–11	6	0	3
B—Subacute and Chronic	6	24–51	0	6	1	2	1	2	200–600	9.1	15–81	38	4	2
Total	9	.....	1	8	1	2	1	5	.....	...	.....	..	4	5
PSORIASIS	4	40–53	4	0	1	2	1	0	200–600	15.4	20–197	52	2	2
SCLERODERMA	3	42–65	1	2	0	1	0	2	200–400	14.1	15–150	96	2	1
CHORIORETINITIS	1	45	1	0	.	.	.	.	400	29.6	74	74	1	0
ERYTHEMA NODOSUM	1	15	0	1	.	.	.	.	400	7.6	19	19	0	1
Total Collagen Diseases (excl. Arthritis)	18	.....	7	11	.	.	.	.	.....	...	.....	..	9	9
ENDOCRINE CONDITIONS														
Malignant Exophthalmos	5	35–66	2	3	1	2	2	.	100–600	17.2	21–150	56	5	0
Addison's Disease	6	26–58	2	4	.	2	4	.	100–300	4.7	4–167	53	0	6
Hyperthyroidism	1	11	0	1	.	.	.	.	100	3.0	30	30	0	1
Myxedema	1	45	1	0	.	.	.	.	200–400	6.8	23	23	0	1
Chronic Cystic Mastitis	1	31	0	1	.	.	.	.	50–300	21.3	180	180	1	0
Obesity	1	54	0	1	.	.	.	.	100–400	11.1	90	90	0	1
Total Endocrine	15	.....	5	10	.	.	.	.	.....	...	.....	..	6	9

TABLE III—*(Concluded)*

CLINICAL APPLICATION OF PREGNENOLONE

Disease and Sub Group*	No. of Cases	Age Range (Yrs.)	Sex M	Sex F	Severity I	Severity II	Severity III	Severity IV	Dosage Daily Range (mg.)	Dosage Total Aver. (Gms.)	Dosage Duration of Range	Dosage R (Days) Aver.	Result Moderate to Marked Improvement	Result None to Slight Improvement
MISCELLANEOUS CONDITIONS														
Hodgkin's disease	1	46	0	1	.	.	.	.	300	6.0	20	20	0	1
Chronic Pancreatitis	2	40–58	1	1	.	.	.	.	150–300	19.2	21–92	56	0	2
Tuberculosis of the Spine	1	41	1	0	.	.	.	.	100–400	5.5	30	30	0	1
Amyotrophic Lateral Sclerosis	1	49	1	0	.	.	.	.	400	56.0	240	240	1	0
Epilepsy	1	15	1	0	.	.	.	.	300	2.4	8	8	0	1
Total Miscellaneous	6	.....	4	2	.	.	.	.	.....	...	.....	..	1	5
GRAND TOTAL	115	.....	42	73	.	.	.	.	.....	...	.....	..	54	61

* Severity—in the arthritics refers to their functional capacity in relation to the classification of the New York Rheumatism Association. (See Ref. 22); the arthritic sub-groups are similarly arranged according to the progression of the disease (Ref. 22). In malignant exophthalmos, severity, grade I represents incipient stage; grade II, ingravescent stage and grade III, malignant stage. Grading of severity is purely arbitrary in terms of degree I to IV in the remainder of the diseases.

standard for measuring the usefulness of the other pregnenolone derivatives. However, our experience with 21-acetoxypregnenolone, 17α-hydroxypregnenolone and pregnenolone betaine hydrochloride is very limited, representing in each instance short periods of administration to not more than a dozen subjects. For these reasons, no details of such studies will be given. Nevertheless, we feel that these preparations are sources from which effective pregnenolone-like action can be obtained. In the present state of our investigations we see neither advantages nor disadvantages to their selection as compared with pregnenolone or pregnenolone acetate, unless certain physical or chemical properties with which we are not acquainted make their preparation easier and/or more economical.

In general, the first evidence of improvement in patients using pregnenolone appeared between the second and fourth days of treatment provided doses of the steroid were sufficiently large. The initial change can best be described as a sense of well being. When analyzed, the patient's statement "I am better" appears to indicate an improved mental outlook and an increase in both mental and physical vigor. In association with this change, there was commonly a marked improvement in appetite; this was sometimes observed on the first day of therapy. That this was not a purely psychologically initiated change seems to be indicated by the fact that it did not occur when placebos were used prior to the initiation of the pregnenolone therapy. Between the fourth and the eighth days of treatment, alterations of a specific nature could be observed in those patients who suffered from a disturbance involving the collagen containing tissues. In the arthritics, there was a lessening of articular and periarticular pains with an increased mobility. In the patients with scleroderma, the sense of "tightness" was lessened and movement of the parts became possible. In subjects with subacute and chronic lupus erythematosus, a diminution in the fiery red color of the lesions could be noticed. In psoriasis, scaling decreased and lesions began to fade. As a rule, a favorable progression of manifestations, if obtained at all, continued for three or four weeks, after which little further improvement occurred, unless the dose of the drug was materially increased. In such instances further improvement might or might not occur and was seen in approximately one-half the subjects so treated. As a rule, the general sense of well being and the good appetite attained early were maintained throughout the course of therapy.

Fever, if present, was usually influenced favorably by pregnenolone and came to normal as a rule between the seventh and fourteenth days of treatment. If affected at all, the sedimentation rate was slightly lowered during the first ten days of therapy, but often returned to the pretreatment level later despite clinical improvement.

In underweight subjects, a gain in weight was usual. Those who were over weight as a rule failed to lose, unless their intake was voluntarily curtailed.

An early drop in the eosinophil count was observed in about 30 per cent of the patients, but was rarely maintained beyond the second week

of treatment. With an increase in the dose of pregnenolone a second fall of similarly transient nature sometimes occurred. A transient rise in 17-ketosteroid excretion followed the use of pregnenolone with a return to pretreatment levels within 6 to 10 days.

As a rule, a maximum therapeutic response was attained not later than the fourth week of therapy; this could usually be maintained by the continuation of therapy and sometimes improved by increasing the dose. In a few subjects, the initial good effects seemed to be lost after a period of ten days to two weeks of therapy. Such patients have been classified as failures and we have been inclined to attribute much of the initial change in them to a psychic influence.

As soon as a maximum response was obtained in any given subject, effort was made to decrease the dose slowly over a relatively long period of time. In about one-third of the patients, remissions of from one to several months were thus obtained. In others, there was a return of symptoms as soon as the amount of steroid used was materially curtailed.

Considerable differences exist amidst the overall and specific responses of the various diseases to therapy with pregnenolone and these may be considered in detail with profit.

I. Arthritis. This group of patients includes 40 with rheumatoid arthritis (including one subject with Still's disease and two with ankylosing spondylo-arthritis), 31 patients with osteoarthritis, 3 with gouty arthritis, one with allergic hydrarthrosis, and one with acute infectious arthritis. All of these were in an active stage of the disease at the time of treatment. In Table III, the classification of the New York Rheumatism Association[22] has been employed for diagnostic, functional and therapeutic purposes.

Twenty of the 40 patients with *rheumatoid arthritis* were moderately to markedly improved by the use of pregnenolone. In stages I and II all of the changes present were usually reversible, *i.e.*, if the patient responded at all. There was no method by which a favorable response could be predicted a priori. Nor did the "severity" of the lesions (degree of functional incapacity) indicate the extent or the permanence of the restitution attained. As far as we could observe, however, where destruction of cartilage or bone had occurred (stages 3 and 4), there was no evidence of a favorable alteration in the function of such joints, although pain and periarticular manifestations might be relieved. As a rule, it was necessary to employ not less than 300 milligrams of pregnenolone or pregnenolone acetate daily to obtain a favorable response and in some instances this had to be increased, at least for a time, to 600 milligrams per day. We have not exceeded this dose in our routine work, so cannot say that further increments will not have an additional effect, though we are inclined to doubt it.

The length of time one should continue the dose associated with maximum improvement is variable. Sometimes reduction may be begun at the end of the first week; in one instance we employed a daily dose of 600 milligrams for 89 days, after which the patient had an apparently permanent remission.

We have had an opportunity to try cortisone in 6 and ACTH in 4 of our failures or partial failures with pregnenolone in rheumatoid arthritis. Cortisone in 100 milligram doses daily helped or further helped two of these and failed to further improve two more. Cortisone 30 milligrams daily and insulin 20 units daily as a combined injection afforded further improvement in the two remaining cases. In both of the patients in whom cortisone and pregnenolone failed to give relief, ACTH was of some value in one and completely relieved the other. Pregnenolone has not helped a subject in whom ACTH completely failed, but seems to have reenforced the action in two subjects upon whom a combined therapy was tried. Pregnenolone has relieved one of three subjects not relieved by cortisone and has given equally good results in one patient with Still's disease.

Sixteen of 31 patients with *osteoarthritis* have obtained moderate to marked improvement from the use of pregnenolone in doses varying from 100 to 600 milligrams daily (Table III). In eight of the failures cortisone has been tried with no better results than those attendant upon the administration of pregnenolone. ACTH has been unsuccessfully employed in two subjects who failed to respond to either cortisone or pregnenolone and has afforded no further relief. Conversely, pregnenolone has been of little help in these subjects who failed to respond to ACTH or cortisone. In general, the pain of the milder forms of osteoarthritis has responded very well to pregnenolone, while there has been little or no change in either subjective or objective symptoms beyond the relief of periarticular spasm and muscle pain in those subjects who have advanced joint lesions.

Of the three subjects with *gout,* one developed a dramatic response to the administration of pregnenolone. A wide variety of medications, including colchicine, had previously failed to relieve his chronically incapacitating lesions. By the third day of pregnenolone therapy, 300 milligrams daily, the joint pains were completely gone, and the patient was able to resume his former occupation. Later, auricular tophi softened and disappeared. Levels for uric acid in the blood were uninfluenced by the drug. The patient has remained symptom-free for nearly a year while taking maintenance doses of pregnenolone. Cortisone subsequently helped one of the other two patients who was only slightly relieved by pregnenolone and failed to improve the third patient. The third subject obtained considerable relief from injections of 300 milligrams of an aqueous suspension of pregnenolone acetate but discontinued them because of pain and induration at the site of injection. He was not further improved by cortisone.

One patient with *acute polyarticular arthritis* of undetermined etiology was relieved of a majority of the joint symptoms during the first five days of a 27-day course of therapy. Concomitantly there was a gain in body weight, an increase in hemoglobin toward normal and a disappearance of fever. A moderate leukocytosis and marked elevation of the sedimentation rate were unaffected by the therapy.

One patient with *allergic hydrarthrosis* was not relieved by pregnenolone.

II. Lupus Erythematosus (Table III). Nine patients with lupus erythematosus have been treated with pregnenolone. Of these, three were of the *acute* variety, and were not relieved by large doses of pregnenolone —up to 600 milligrams daily. Cortisone, subsequently employed, afforded some relief to two of the three. ACTH brought about a temporary remission in this third subject and still further improved the condition of one of those partially relieved by cortisone.

Among the six patients with *subacute* or *chronic lupus erythematosus,* one case of the discoid variety has been included. It was not improved by therapy with pregnenolone. Of the remaining five, four were moderately to markedly improved by pregnenolone, but in no case did the skin lesions completely disappear. In all instances, the systemic manifestations were relieved. A change for the better in mental outlook and in muscular strength were particularly discernible.

III. Psoriasis. Four patients with psoriasis, two of whom also had arthritic lesions, have been treated with pregnenolone. The two subjects who had the more widespread lesions and in whom arthritis was present responded well to pregnenolone. One of these patients had been treated with a wide variety of medications for two years prior to the use of pregnenolone and had shown little or no alteration in his condition. This plus the fact that he has been kept practically free of manifestations of his disease for about eight months while taking 300 milligrams of pregnenolone daily minimizes any psychic effect the use of the drug could have had. The other subject with marked improvement was treated for approximately seven weeks, and has had no exacerbation of his lesions in the seven months which have since elapsed.

IV. Scleroderma. Two patients with widespread sclerodermatous lesions and one with involvement localized to the cheeks and dorsal surfaces of the forearms have been treated with pregnenolone in daily doses ranging from 200 to 400 milligrams. The last mentioned patient obtained complete relief with pregnenolone.

One of the other patients with practically complete fixation of the skin of the face and hands and with a severe completely incapacitating arthritis of the lower extremities was markedly improved with pregnenolone in doses of 400 milligrams daily. Through its use she became capable of feeding herself and with the aid of a chair or crutch was able to take a few steps. Whenever the drug was stopped, her condition rather promptly relapsed. Cortisone 30 milligrams and insulin 20 units daily were tried during such a relapse and afforded her approximately the same amount of relief as 400 milligrams daily of pregnenolone.

The third subject with a severe form of scleroderma had a practically completely fixed chest wall so that for years his breathing had been entirely abdominal in type. Pregnenolone afforded him slight improvement, but not as much as a small dose of cortisone (30 mg. daily) in conjunction with insulin (20 units daily).

In treating scleroderma, the maximum softening of the skin and sub-

cutaneous tissues was achieved between the third and fifth weeks of treatment and could be maintained as long as the therapy was continued.

V. Chorioretinitis. One patient with chorioretinitis of undetermined etiology was given 400 milligrams daily of pregnenolone for 74 days with nearly complete clearing of the condition. However, as the condition had already begun to improve prior to the initiation of steroid therapy, no credit is given its action in this case (Table III).

VI. Erythema Nodosum. No clinical benefit was observed in one subject with erythema nodosum who received 400 milligrams of pregnenolone daily for 19 days (Table III).

VII. Addison's Disease. Six patients with demonstrable Addison's disease were given pregnenolone in some form in daily doses ranging from 100 to 300 milligrams for periods of time varying from 4 to 167 days (Table III). The mildest of these cases stated that she felt better while taking the steroid, but despite this fact continued to lose weight until other medications were added. None of the other patients showed any subjective or objective improvement and one of them actually stated that he felt worse as a result of the therapy. He complained of an increased incidence of headaches with dizziness and nausea, all of which he attributed to the pregnenolone.

One patient, who was losing weight rapidly at the time the administration of pregnenolone was begun and who was at that time approaching crisis, continued to lose and showed little change in her negative balance for nitrogen while taking the steroid (Fig. 8).

VIII. Malignant Exophthalmos. In four of five patients with malignant exophthalmos, the onset of the disease followed thyroidectomy for hyperthyroidism (Table III). One of these was in the incipient stage and had an exophthalmos of only 22 mm. Two of the remainder were in the ingravescent stage, with more marked exophthalmos, while the remaining two were bordering on the malignant phase of the disease with exophthalmos above 30 mm. in both instances. In all five of these subjects, there was marked improvement which began on the second to fourth days of treatment. By the fifth day, there was a decrease in periorbital edema, conjunctival reaction and tearing associated with less difficulty in closing the eyes. Two of the severest cases and one of the moderately severe showed a diminution in the exophthalmos varying from 2 to 5 millimeters.

IX. Hyperthyroidism. Because of the dramatic relief observed in subjects with exophthalmos, one 11-year-old subject with a forme fruste type of hyperthyroidism was given pregnenolone without influencing the course of her condition in any way (Table III).

X. Obesity. In view of the tendency for pregnenolone to produce a slightly negative balance for nitrogen, the weight of several of our obese subjects with osteoarthritis was observed closely. In none of these, nor in one person with an exogenous obesity did the drug seem to cause any reduction in weight (Table III).

XI. Chronic Cystic Mastitis. Pregnenolone completely relieved pain

in the one individual with chronic cystic mastitis to whom it was administered. Following 21 days of therapy with a daily dose of 300 milligrams, the amount was reduced to 50 milligrams daily. By the end of the fourth month not only had all pain disappeared but "lumpy areas" could not longer be palpated in the breast. Furthermore, extreme lassitude of which the patient had complained for several years was replaced by a feeling of vitality and well being.

XII. Myxedema. In view of some of the relationships which have been described as existing between the thyroid and the adrenal cortex, pregnenolone was tried unsuccessfully in a typical case of myxedema whose outstanding complaints included a coarse, harsh, dry, thickened skin and vague, more or less generalized joint pains with cracking on motion (Table III).

XIII. Miscellaneous Conditions. We have employed pregnenolone in several totally unrelated conditions chiefly with a view to determine whether or not its nonspecific analgesic effects could be elicited by doses commonly used in human beings (Table III). No relief was afforded those who suffered from the itching of Hodgkin's disease, the pain of chronic pancreatitis or the pain of tuberculosis of the spine.

The appetite of a patient with amyotrophic lateral sclerosis was markedly improved and his nutritional status favorably influenced by the use of 400 milligrams of drug daily. There was a sufficient return of muscular strength in the involved interossei to enable him to resume his work as an artist.

Toxic Reactions to Pregnenolone

In the course of administering pregnenolone to 115 subjects in daily dosages ranging from 100 to 1000 milligrams, side effects have been described in three instances. One of these complained of dizziness after each injection. A second was nauseated following the use of the drug by mouth and occasionally "felt dizzy." The third discontinued the drug because it made him "nervous" and kept him awake. None of these three subjects was given a maximal dose of the drug. Unfortunately placebos were not tried in any of them. Local reactions to intramuscular injections of pregnenolone and pregnenolone acetate occur occasionally whether the medium is water or sesame oil. Rarely, however, do they necessitate stopping the drug. We have seen two instances of sterile abscess developing at the site of injection, both of which healed very slowly. These patients had received the drug intramuscularly for considerable periods of time in doses of 300 milligrams twice daily. Cohen, Goldman and Dubbs[7] say these can be avoided by aspirating a "suspect" area with an 18 gauge needle.

Discussion

It appears that pregnenolone is a useful drug in the management of the so-called collagen diseases and is capable of producing sustained remissions in a significant number of cases. It is equally apparent that it is

not as effective against this group of diseases as is cortisone or ACTH, with the difference between it and cortisone less marked than between it and ACTH.

1. Action of Pregnenolone in Arthritis

By far the largest amount of reported work with pregnenolone has been concerned with its application to rheumatoid arthritis, as aside from the present studies, we have been able to collect 522 cases reported by 18 observers[1–18, 23] (Table I). Four observers[1, 2, 3, 4] who were not impressed with the activity of the drug employed doses of 200 milligrams daily or less in a majority of their patients except for short periods of time. In much larger doses in a small group of cases one of these workers[3] noted temporary improvement. Of the 14 investigators who have considered the drug useful, three[8, 17, 18] have observed consistently good effects in a significant percentage of their patients when doses of 100 milligrams or less have been administered daily. However, one of these[18] used the compound always in conjunction with other steroid hormonal therapy, so that no effort was made to appraise its effectiveness when used alone. In the 522 cases included in the 18 reports above mentioned, 272 or 52.2 per cent are stated to have obtained some degree of improvement. Major improvement has occurred in 20 of the 40 patients with rheumatoid arthritis (Table III), and it has been possible to maintain fifteen of these in remission. What distinguishes the responsive from the unresponsive individual is not clear, but the role of psychic influence in our subjects seems to have been minimized by the liberal use of placebos both before and during therapy.

Cortisone frequently helps subjects with rheumatoid arthritis who are not relieved by pregnenolone, but it rarely gives any greater degree of improvement in those who have been improved by the latter preparation, although its effects may be evidenced more quickly and dramatically. Side effects and cost are drawbacks to the widespread use of cortisone, although most of the side effects can be avoided if we are not led astray by the earlier work in which much larger doses than are usually necessary were used. Many patients respond well to 50 milligrams daily. Perhaps suppression of adrenal and pituitary activity as a result of long continued use needs most to be feared. ACTH appears to be more active against rheumatoid arthritis, as indeed against the other so-called collagen diseases, than either pregnenolone or cortisone. However, it too produces many, if not all, of the side effects attendant upon the employment of cortisone in addition to which the problems of diminishing reactivity and developing sensitivity must be added. A considerable tendency has arisen to favor the use of one of these agents to the exclusion of the other two; in daily practice there seems to be a place for all three.

While 16 of 31 patients with arthritis responded in some measure to pregnenolone, our ability to afford such subjects complete relief was slight. Cortisone and ACTH appear to be little if any more effective. From the present series of observations it is suggested that these prepara-

tions act not upon the primary condition within the joint as they may do in early rheumatoid arthritis, but only upon disturbances within the surrounding muscular and fibrous tissues. At least, they seem to relieve spasm, pain and swelling in the periarticular soft tissues, but do not improve the condition of the joint surface; hence, complete relief of pain and incapacity, except in the earliest cases, is not attained. The "wearing off" effect seen in osteoarthritis may be related to these facts.

2. The Metabolic Action of Pregnenolone

The tendency for pregnenolone to induce a negative balance for nitrogen is modest but definite and the temporary storage which follows its withdrawal is probably statistically significant. However, the steroid can cause nitrogen retention if the protein intake is sufficiently increased irrespective of whether this is in response to an increased appetite as induced by the drug itself or secondary to a metabolic need as might occur in pregnancy.

Changes in the electrolyte pattern of excretion are on less solid ground. In the present study there has been a tendency toward an increased excretion of sodium, chloride and potassium but this has been slight and showed wide fluctuations from subject to subject and from time to time during the administration of the steroid. If salt intake is restricted, there was in each of four subjects a tendency to retain sodium and to a lesser extent, chloride. These changes are minor but may suggest a variation in the action of the drug as conditioned by the intake of salt. All of the changes in electrolytes have been minor ones and caution should be observed in attempting to attach too much importance to them.

An increase in the excretion of the 17-ketosteroids occurred in conjunction with pregnenolone administration and was reversed by the stress imposed by a low intake of salt. Nevertheless, these changes were slight and the observations too few to assume statistical significance. In other circumstances associated with stress, the administration of pregnenolone lowered the urinary values for this group of steroids.[24, 25, 26]

3. The Effects of Pregnenolone When Administered to Subjects with Endocrine Conditions

The failure of pregnenolone to influence materially the clinical picture of Addison's disease and its inability to alter nitrogen balance in one such patient led to the thought that the adrenal cortex might be concerned in mediating some if not all of its action. If this were true then subjects with a presumably intact adrenal might exhibit alterations in pituitary and thyroid activity following its use.[27, 28] Because of this and the collagenous tissue changes to be observed in the ophthalmopathic form of Graves' disease, the steroid was administered to five subjects with severe exophthalmos with dramatic relief of the subjective symptoms and objective changes in the exophthalmos in the severest cases.

The failure to influence acute lupus erythematosus is less easily explained on the basis of adrenal function alone, particularly as stimula-

tion of the adrenal by ACTH has yielded complete, albeit often very temporary, relief in this condition. It may be that the need of the tissues for adrenal cortical hormones is not sufficient stimulus in this condition to excite the pituitary-adrenal system and that such a stimulus can be found in an intermediary product of adrenal metabolism such as pregnenolone may be.[29, 30] Although no theory seems perfectly to fit all the facts, it is suggested that a functioning adrenal may be necessary for the conversion of pregnenolone to an active hormonal material capable of favorably influencing a group of diseases in which disturbances of mesenchymal tissues are primarily involved. In any event pregnenolone has afforded sufficient relief to sufferers from diseases associated with alterations in the connective tissues of the body to warrant not only extended trial but also a search for more active and equally nontoxic compounds.

Summary

1. Pregnenolone or its acetate has been administered orally or parenterally to 132 subjects, of whom 17 were selected for certain metabolic studies and the remainder treated for a wide variety of diseases.

2. Pregnenolone in aqueous suspension injected intramuscularly caused an increase in nitrogen excretion which could be reversed by increasing the nitrogen intake. Sodium, potassium and chloride excretion was furthered, but this action was transient and variable in degree. There was a tendency for it to be reversed when the intake of salt was low.

3. A slight transient increase in the excretion of 17-ketosteroids was noted in conjunction with the administration of pregnenolone. A low salt intake tended to efface or reverse this effect.

4. The active inflammatory factors concerned in the collagen diseases were favorably influenced by pregnenolone except in the case of acute lupus erythematosus, which did not respond at all. Of 78 patients with some form of arthritis, 38 or 50 per cent were moderately or markedly improved. More than half of the cases of chronic lupus erythematosus, psoriasis, scleroderma and erythema nodosum were favorably influenced by the drug.

5. No improvement was noted from the use of pregnenolone in Addison's disease.

6. All of 5 patients with ophthalmopathic Graves' disease obtained symptomatic relief from the use of pregnenolone and in the 3 most severe there was a measurable recession in the exophthalmos.

7. The literature dealing with the administration of pregnenolone in rheumatoid arthritis is briefly summarized.

8. The nature of pregnenolone activity is discussed.

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Discussion

Discussion on this paper follows Chapter 20.

19

Studies on the Clinical, Metabolic, Electrolyte, Hormonal, and Immunologic Effects of Δ^5-Pregnenolone in Rheumatoid Arthritis. Interaction of Cortisone, ACTH, and Pregnenolone

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There is no doubt that as a result of the work of Hench and his associates[1, 2] on the significantly beneficial effects of cortisone and ACTH in some rheumatic diseases, a new era began in the investigation and treatment of such pathologic conditions.

Since our present knowledge of rheumatology and the mechanism of action of the abovementioned hormones does not permit us to consider rheumatoid arthritis and the rest of the collagenous diseases as endocrinopathies, in which cortisone and ACTH could be the specific therapeutic agents, it may be speculated that other hormones, viz., steroids and similar substances, may play a favorable role on the clinical course of such conditions.

After a screening of various steroid compounds, pregnenolone was selected to try on a larger scale in patients with rheumatoid arthritis. Inasmuch as it is of interest to record not only the clinical course of a given disease but also its mechanism of action, we conducted laboratory studies on electrolyte, protein, lipid, and carbohydrate metabolism, as well as assessing hormonal and immunologic effects during the administration of the steroid.

Our results have been indicated in a preliminary report[3] published in August, 1949. The presentation here deals with follow-up studies of those patients for periods of many months and gives the complete data of the results obtained to the present time.

We have also conducted a study of the therapeutic interaction of pregnenolone, cortisone, and ACTH, judged by the clinical effects on patients with rheumatoid arthritis treated alternatively or simultaneously with combinations of these hormones.

Material and Methods

Experiment 1. A selection was made of 31 patients with rheumatoid arthritis of varying degrees of activity and duration. They were chosen mainly on the basis of having active rheumatoid arthritis with a degree of stability in the course of the disease.

The general characteristics of the patients and their rheumatoid arthritic condition were as follows: Average age, 35 years, 8 months, 12 days; the oldest patient was 64 years and the youngest 4 years; 25 patients were females and only 6 were males; average duration of the disease was 4 years, 8 months, and 22 days; maximum duration, 15 years, minimum, 10 months.

The severity of the disease was classified in four different degrees; 1 patient was in group I; 17 in group II; 11 in group III, and 2 in group IV. In relation to the degree of activity of the disease, there were 2 patients in group I, 17 in group II, 11 in group III, and 1 in group IV. With reference to the degree of activity of the rheumatoid arthritis at the time of this study, in relation to its former evolution, there was an increase in 4 cases, improvement in 4 others, and the remaining 23 had long ago attained a certain degree of stability. The general condition of the patients was good in 5 cases, fair in 19 and bad in the remaining 7. The criteria for evaluating the severity and activity of the rheumatoid arthritis followed as closely as possible the specifications of the American Rheumatism Association, as published by Steinbrocker, Traeger, and Balterman.[4]

In all the cases, the diagnosis of rheumatoid arthritis was established following clinical studies and with the aid of necessary laboratory and x-ray examinations. All of the patients presented a rather characteristic clinical picture. In some cases there was involvement of the spine as well as peripheral joints, but none of the patients had reached the stage of true rheumatoid spondylitis.

The patients were divided into two groups. The first group represented 28 patients who undertook treatment without hospitalization, but were subject to a clinical examination every two or three days. In addition, this group was checked every eight days for a sedimentation rate determination, and a blood count was done every eight days. Streptococcus agglutination tests were conducted at intervals. Roentgenologic studies were done before and during treatment.

The data were recorded on previously prepared charts, with respect to pain, joint swelling, limitation of movements, stiffness, and muscular strength. The data gathered include even the slightest variation of these signs and symptoms. The general condition, weight, and subjective sensations were also analyzed.

The second group in this study was comprised of two patients, belonging to the originally studied group of 31, plus a patient with rheumatoid spondylitis. These patients were hospitalized and underwent the same studies as the members of the first group. Besides these studies

mentioned above, the following were determined: urinary and serum sodium, potassium, chloride, urea, uric acid, and creatinine; blood glucose; plasma proteins; serum cholesterol; glucose and insulin tolerance tests; urinary 17-ketosteroids, pregnandiol, and estrogens; eosinophil counts; and the water test. Most of these determinations were conducted two times each week during pregnenolone administration.

All patients suspended all previous treatment for some days prior to the hormonal therapy. The latter consisted of the administration of pregnenolone and pregnenolone acetate in aqueous suspension or in oil solution, in doses of 100 to 200 mg. per day intramuscularly. After 10 to 15 days of treatment, undesirable tissue reactions made it necessary to administer the steroid by oral route in doses of 200 to 400 mg. per day. The average treatment period was two months and seven days. The maximum period was four months and the minimum was two weeks.

Experiment 2. A group of patients with rheumatoid arthritis was studied in order to investigate the possibility of a synergism of pregnenolone with cortisone or with ACTH. Four patients were treated on alternate days with 100 mg. of cortisone and 300 mg. of intramuscular pregnenolone in oil solution for periods of 10 days each. The clinical immunologic, metabolic, and hormonal results were analyzed as in the previous study.

The same procedure was carried out on three different patients, using ACTH instead of cortisone. The dosage of ACTH used was 80 mg. given in four injections, one every six hours, every other day. On alternate days, 300 mg. of pregnenolone was injected. Three additional patients of this group were treated with pregnenolone and ACTH or cortisone together daily, for periods of 15 to 20 days.

Another patient was treated with ACTH alone, and after a favorable clinical response, the dosage was lowered each four to five days until the appearance of joint symptoms. This occurred when the daily dose of ACTH had been decreased to 30 mg. At that time, 300 mg. of pregnenolone was administered and the clinical results were carefully recorded during the following 10 days. Three additional patients were treated in the same way, using cortisone instead of ACTH.

Results of Experiment 1. The results observed after the use of pregnenolone in the 31 patients with rheumatoid arthritis, can be divided into two types of data: (1) the clinical effects, and (2) the metabolic (electrolyte, protein, lipid, carbohydrate, hormonal) effects.

The clinical results were based on the specifications of the American Rheumatism Association for judging the therapeutic action of different drugs. The effects were classified in degrees from slight (I) to complete remission (IV). It was found that 18 patients had an improvement of grade I; 5 were in the class II degree of improvement; 4 patients showed no improvement; and 4 became worse in grade I. In order to be more precisely aware of the improvements, it is necessary to have a detailed account of their characteristics and evolution. This recording is of the utmost importance inasmuch as when only round figures are recorded,

one may get an incorrect impression regarding the action of the drug studied. Furthermore, it should be kept in mind that although rheumatoid arthritis is a chronic disease, it may have fluctuations. It is convenient to compare the results obtained with the administration of pregnenolone with those obtained after the administration of other therapeutic agents.

In Fig. 1 is presented the course of the arthritis in 31 patients treated with pregnenolone. In those patients affected favorably, the improvement consisted of one or more of the following developments:

FIG. 1. Evolution of the arthritis in 31 patients with rheumatoid arthritis treated with pregnenolone.

1. A very slight decrease of the articular phlogosis, appearing toward the end of the second week or during the course of the third. It was not strikingly significant and vanished after one to two months, leaving almost all of the patients with the same degree of joint phlogosis at the end of treatment as was present at the beginning.

2. A slight decrease of the articular pain, evident in most cases during the second week of treatment. This improvement was observed during several weeks although it showed a tendency to vanish.

3. A slight increase in the range of the articular movements so that they could be performed more easily and strongly. This phenomenon appeared, also, more or less between the second and third weeks. The patients also observed a decrease of articular stiffness as well as an increase of muscular strength.

4. A slight favorable effect of the general condition. Furthermore, the patients described a sensation of wellbeing and showed some euphoria and optimism. Nevertheless, in many of the cases such states promptly disappeared and were replaced by a feeling of depression. Table I pre-

TABLE I

DETAILED EXAMINATION OF JOINT CHANGES IN A PATIENT WITH RHEUMATOID ARTHRITIS, WHO SHOWED GOOD RESPONSE TO PREGNENOLONE THERAPY

Joint	*Joint Swelling*		*Synovial M. Thickness*		*Synovial Fluid*		*Pain*		*Tenderness*		*Limitation of Motion*		*Circumference Measurements*	
	B*	A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B* Cm.	*A* Cm.
Temporomaxillary	−	−	?	?	−	−	+	−	−	−	10%	5%		
Cervical column	−	−	−	−	−	−	+	±	−	−	30%	15%		
Right elbow	++	±	+	±	+	−	+	±	+	−	10%	5%	18	16
Left elbow	+	−	+	−	−	−	−	−	−	−	−	−	16	16
Right wrist	++	+	++	++	+	−	+	+	+	−	100%	100%	16	16
Left wrist	++	+	++	++	+	−	+	+	+	−	100%	90%	16	16
R. Metatarsophalangeals	+	+	+	+	−	−	+	+	−	−	90%	50%		
L. Metacarpophalangeals	+	+	−	−	−	−	+	+	−	−	70%	10%		
R. Interphalangeals	+	+	+	+	−	−	+	−	−	−	60%	15%		
L. Interphalangeals	−	−	−	−	−	−	−	−	−	−	−	−		
Coxofemorals	?	?	?	?	?	?	++	+	−	−	70%	50%		
Right knee	+	−	+	−	+	−	+	−	−	−	10%	10%	26	25
Left knee	−	−	−	−	−	−	−	−	−	−	−	−	−	
Right ankle	++	+	++	+	+	+	++	+	+	−	100%	90%	25	23
Left ankle	+	+	+	+	−	−	+	−	−	−	100%	90%	21	20
Metatarsophalangeals	+	+	+	+	−	−	+	−	−	−	−	−		

* B, before treatment; A, after treatment.

sents in detail the joint changes in a patient with rheumatoid arthritis who showed a good response with pregnenolone.

The improvement of all the described manifestations was slight but unquestionable, particularly since most of the patients belonged to a low social and economic class, thus suggesting a minimum degree of psychologic influence. All of the patients had been treated several times with various therapeutic agents and they were skeptical about any new drug. The patients were not informed of the favorable course of the investigation.

FIG. 2. Comparative study between the grade of improvement in 31 patients treated with pregnenolone and the grade of activity of the disease.

Considering all the patients in whom we found any degree of subjective or objective improvement, no matter how slight, a beneficial effect was present in 77.0 per cent; only 23.0 per cent showed objective improvement.

The sedimentation rate before and after treatment showed no significant variations. There was a slight decrease in the sedimentation rate in some cases, but others revealed no significant change; fluctuations were so numerous that the data could not be charted conveniently. There was no parallelism between the clinical improvement and the changes in the sedimentation rate.

The blood counts revealed only a slight decrease in the number of leukocytes in 66 per cent of the patients, and in 75 per cent of the patients there was an improvement of their mild anemia. There were no other significant changes in the blood cytology.

The streptococcus agglutination test showed a slight diminution in some of the patients in whom it was high prior to treatment. The relation

of this to the activity of the disease and the beneficial effect of pregnenolone could not be correlated, although there may have been an indication of a positive correlation in those patients showing the greatest activity (Fig. 2).

With regard to the action of pregnenolone on the blood and urinary constituents which were measured, no important changes were seen with the exception of a slightly significant increase in the 17-ketosteroid excretion in a few of the cases.

We did not observe any toxic effect of pregnenolone except that two patients showed a reactional dermatosis at the site of injection. There were no signs of adrenal hypofunction and only slight menstrual disturbances.

FIG. 3. Comparative study of the rheumatoid arthritic condition of 3 patients treated with ACTH and pregnenolone.

Results of Experiment 2. The clinical results obtained in the four patients who were treated alternately with cortisone or ACTH, and pregnenolone, can be seen in Figs. 3 and 4. In all of the cases, there was a marked decrease in the rheumatic manifestations during treatment with cortisone and ACTH. This improvement disappeared during treatment with pregnenolone and reappeared after the readministration of cortisone or ACTH.

In the three patients who were treated simultaneously with pregnenolone and suboptimal doses of either cortisone or ACTH, the course of the clinical picture was slightly different as compared to the patients receiving the hormones on alternate days. In the two cases in which

cortisone was used, the articular manifestations of rheumatoid arthritis could be controlled with doses of cortisone smaller than those generally required. Ten days later the experiment was repeated, without the use of pregnenolone, when the rheumatic manifestations had reappeared and when the cortisone dosage was held at 50 mg. daily. Symptoms persisted for a week; to a greater degree than seen when the cortisone had been supplemented with pregnenolone. This fact was evident in one patient; in the other the result was not so clear.

In the patient treated with ACTH and pregnenolone the rheumatic picture did not seem to be modified by the use of the steroid.

FIG. 4. Comparative study of the rheumatoid arthritic condition in 4 patients treated with cortisone and pregnenolone.

Discussion

From the results of the present investigation it would appear that pregnenolone can have a favorable effect on the clinical course of some of the rheumatoid arthritis patients. Since the improvement observed was slight and temporary, there remains the question of whether such favorable trend does not correspond to the usual fluctuations in activity of rheumatoid arthritis. We are inclined to question the improvement even more on the basis of being unable to observe an evident or constant effect on certain tests that indicate either rheumatic activity or the immunologic process of the disease, e.g. erythrocyte sedimentation rate, streptococcus agglutination, etc.

In any event, pregnenolone may well have a pharmacologic action; this is concluded because of the following reasons: The patients studied

were selected on the basis of the stability of their rheumatoid arthritis. All had been suffering with the disease for many years and the pathologic processes were firmly established. During treatment almost all of the patients suffered no change in their usual way of life. Therefore, it appears that there was no apparent psychic or physiologic explanation for the observed improvement, and it is difficult to accept the idea of a spontaneous improvement arising during the natural course of the disease. This is particularly true because of the special pattern followed by the improved patients and also if one considers the high percentage showing some degree of improvement.

The degree of improvement in 77.0 per cent of the patients treated with pregnenolone was slight. It is worthwhile, therefore, to establish comparisons between the effects of other therapeutic procedures and those of pregnenolone, so that the physician may have fair standards with which to judge the usefulness of this steroid.

Most rheumatologists agree on the beneficial action of gold and of a conservative treatment consisting of rest, good dietary regimen, analgesics, and physiotherapy. Through the personal experience of two of the authors and the published works on the therapeutic action of the above-mentioned procedures, it is possible to establish a comparison between them and the effects of pregnenolone. On this basis one can conclude that the results with pregnenolone are very similar to those with the other therapeutic procedures in use insofar as the degree and intensity of the improvement are concerned. There is, however, a difference in the time of appearance, and the duration, of the improvement. If the effect with pregnenolone is compared with the action of cortisone, or with that of ACTH, described by various authors, there is obviously a great difference not only in the degree of improvement, but also in the percentage of rheumatoid arthritis patients showing improvement. It is possible to rate the degree of improvement reached with cortisone and ACTH as III and IV.

We cannot draw conclusions regarding the delayed or prolonged effect of pregnenolone or compare it with results produced by other drugs or hormones since the results observed were short-lived, and the experimental conditions and responses varied with the mode of administration. This might be the cause of the decrease or disappearance, after several weeks, of the clinical improvement observed at the beginning of treatment.

It is difficult to analyze the results of Experiment 2 because in the first place the number of patients treated was very small, and second, because the experiment was conducted for a rather short duration of time. It is possible that the results would have been different if the administration of steroid had continued for longer periods. We may conclude that apparently pregnenolone does not have a synergistic action when used for short periods of time alternately with cortisone or ACTH.

Since the time of our first report,[3] there have appeared in the medical literature several investigations of the use of pregnenolone in rheumatoid

arthritis.[5, 7] The results, although contradictory in some respects, have indicated the value of further studies of this steroid.

Our present knowledge is not extensive enough to allow the discussion of the reasons why the patients with rheumatoid arthritis treated with pregnenolone did not show a decrease in erythrocyte sedimentation rate concomitant with the clinical improvement, since this occurs with most of the efficacious drugs. It is also difficult to speculate regarding the mode of action of pregnenolone, inasmuch as apparently it does not have any special metabolic action, which would permit some explanation, e.g. stimulation of the adrenals and a greater elaboration of corticosteroids. The eosinophil data do not support the suggestion of a direct action on the adrenals. It would be of interest to experiment with this steroid on the enzymatic processes of the organism, and to examine its action on hyaluronidase and certain oxidation-reduction systems. Perhaps such studies may permit us to obtain useful knowledge that could help to explain the apparent beneficial effect of pregnenolone.

Summary and Conclusions

Thirty-one patients with rheumatoid arthritis were treated with pregnenolone and studied from the clinical standpoint. In three patients of this group, the electrolyte, metabolic, and hormonal balances were studied.

The dosage of pregnenolone used was 100 to 200 mg. per day by intramuscular injection or 300 mg. per day orally. The possible mechanisms of action of the steroid studied were discussed and the results obtained through its use were evaluated and compared with those seen following the administration of other drugs or hormones of known activity.

The conclusions reached were the following:

1. The use of pregnenolone had a slight but favorable action on 77.0 per cent of the 31 patients studied, based on consideration of all types of subjective or objective improvement of the clinical symptoms.

2. The improvement consisted mainly in a decrease of the articular pain, an occasional decrease in joint swelling, and a wider range of movements. The general condition of the patients was also slightly improved. The improvement observed had a tendency to disappear in the course of some weeks.

3. There were no constant changes in the erythrocyte count, sedimentation rate, leukocytosis, and in the streptococcus agglutination test.

4. No significant effects were observed on electrolyte, protein, carbohydrate, or lipid metabolism, or on hormonal excretions.

5. A synergistic action between pregnenolone and ACTH in one patient and cortisone and pregnenolone in two patients could not be determined clinically after short alternate courses of therapy.

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Discussion

Discussion on this paper follows Chapter 20.

20

Clinical and Biochemical Studies During Pregnenolone Therapy

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The recent advent of more adequate recognition of the role of adrenal cortical steroids in a wide variety of diseases, hitherto considered to be without a therapeutic common denominator, has logically stimulated interest in the possible clinical utility and the associated biochemical effects of congeners. In the studies presented here, pregnenolones* (Δ^5- or 21-acetoxy)† have been administered orally or by intramuscular injection to patients afflicted with a variety of diseases.

Materials and Methods

Δ^5-Pregnenolone acetate has been administered to 8 patients (in 3 *per os* and in the remainder by intramuscular injection) in 235 to 1000 mg. per day dosage for periods up to 3 weeks in length. Two were ill with disseminated lupus erythematosus, 3 had active rheumatoid arthritis, while the remainder were hospitalized for acute rheumatic fever with carditis. The 21-acetoxypregnenolone was injected, with one exception, in 1000 mg. amounts each day into 2 patients with acute leukemia, 3 others with rheumatoid arthritis and into one adult with chronic rheumatic heart disease and severe congestive failure.

Local and systemic effects, if any, have been noted. In addition, in most of the patients complete balance data relevant to nitrogen, chloride, sodium, and potassium exchanges prior to, during, and often following pregnenolone therapy are available, together with measurements of whole blood nonprotein nitrogen and sugar as well as serum cholesterol, bicarbonate, chloride, sodium, potassium, calcium, inorganic phosphate and water concentrations. In all cases where balance studies were done, and in most of the other instances, patients were maintained on calculated and measured milk-carbohydrate-protein formulae. These formulae were sodium-free in instances where normal sodium intake was clinically contra-

* The term "pregnenolones" is not desirable inasmuch as 21-acetoxypregnenolone is actually more closely related to desoxycorticosterone (Ed.).

† We are indebted to the National Drug Co. for generous supplies of these compounds.

indicated.* Whenever possible the customary indices for evaluating the activity of certain classes of steroids (absolute eosinophil counts, creatinine and uric acid excretion, 17-ketosteroid output) have been employed. Finally, the possible influences of these compounds on circulating protein-bound or serum precipitable iodine values, which under most circumstances reflect quite accurately the level of thyroid activity, have been examined.†

FIG. 1. D.H. During treatment of acute rheumatic fever with Δ^5-pregnenolone, the temperature, pulse rate, and sedimentation rate returned to normal, and migratory arthritis and cardiac murmurs disappeared. Toward the end of the first course of therapy, local reactions developed at the pregnenolone injection sites. Withdrawal of therapy was followed by an exacerbation of acute rheumatic fever. Retreatment with larger amounts of pregnenolone resulted in a sterile abscess in the buttocks.

The balance technique and the analytical procedures employed have been described in previous publications from this and other laboratories,[1-5] except for 17-ketosteroids which were determined‡ by the method of Holtorff and Koch,[6] uric acid in urine for which the modified Kern-Stransky technique was used,[7] eosinophil counts performed as de-

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‡ Dehydroisoandrosterone was used as the standard, and was kindly supplied by Ciba.

scribed by Roche.[8] The clinical response has been depicted in a series of figures with attached legends while the biochemical findings are presented in 6 tables.

Results

A. Clinical Findings in Disease States Treated with Pregnenolones. Of the three patients with rheumatic carditis receiving Δ^5-pregnenolone a remission occurred during therapy in D.H. (Fig. 1) and E.W. im-

FIG. 2. E.W. During parenteral Δ^5-pregnenolone therapy, the sedimentation rate decreased, and pericardial friction and prolongation of the P-R interval disappeared. Fever, gallop rhythm, and apical systolic murmurs persisted.

proved to a lesser degree if at all (Fig. 2), while J.M. (Fig. 3) died on the ninth hospital day. Patient M.M., ill with lupus, went into a complete remission during Δ^5-pregnenolone administration. It should be pointed out that a trend toward some measure of spontaneous improvement had been evident earlier (Fig. 4). The other patient with disseminated lupus (L.G.) was treated for a shorter period and with smaller amounts (Fig. 5). Several observers remarked upon the blanching of the eruption but this was not associated with any other change. None of the 3 patients with active rheumatoid arthritis (Fig. 6, none available for F.W. and M.F.) reported any improvement during pregnenolone administration, though in F.W. the sedimentation rate declined toward normal.

The relatively short periods of therapy in patients without discernible improvement should be noted together with the fact that in V.E., D.H., F.W. the injection of Δ^5-pregnenolone resulted in local reactions.

In the case of patients receiving 21-acetoxypregnenolone the dosage was in general larger and more uniform, but therapy usually could not be prolonged because of local reactions to intramuscular injections. In the two patients in whom local reactions were minimal, S.C. and C.V. (Figs. 7 and 8), clinical improvement accompanied administration of this compound, though there is no assurance that it was etiologically related. In

FIG. 3. J.M. In this severely ill patient with active rheumatic carditis, treatment with increasing amounts of Δ^5-pregnenolone i.m. for six days failed to alter the progressive downhill course.

the others, one of whom (F.W.) went on to sterile abscess formation, the disease state was not visibly influenced (Figs. 9 and 10).

B. The Relative Constancy of Blood and Serum Constituents during Therapy (Tables I and II). In general fasting blood sugar values remained below 100 milligrams per cent even though as much as 1 gm. of either of the two steroids was given during intervals up to several weeks in length. The exceptions recorded in Table I were still within the upper limits of sporadic variation and were unassociated with glycosuria. Glucose tolerance studies were not done. Elevations in blood nonprotein nitrogen in patients with cardiac disease were merely additional evidences

of congestive failure; decline toward or to nonelevated concentrations occurred during clinical improvement. In the patient with myelogenous leukemia, W.McF., and in one of the patients with lupus, L.G., the sequence of the data suggests that the azotemia was a manifestation of the primary disease.

There is no evidence to show that either steroid raised the serum bicarbonate level to abnormal values in any patient, nor is there any suggestion of a reciprocal rise in bicarbonate in the one patient in whom hypochloremia supervened during therapy. Fluctuations in serum sodium

FIG. 4. M.M. This 6½-year-old girl with lupus erythematosus disseminatus went into a partial spontaneous remission prior to pregnenolone therapy. Remission became complete during oral pregnenolone ingestion without subsequent exacerbation.

and potassium were quite as great during the control periods as in the intervals of pregnenolone therapy. In neither case is there any trend evident. There were no abnormally low serum potassium levels recorded; the highest potassium value observed, 6.6 milliequivalents per liter in L.G. (Table I) followed KCl therapy. Serum calcium, inorganic phosphate and water concentrations were not detectably influenced by pregnenolone ingestion or injection. Finally, it should be pointed out that the 21-acetoxypregnenolone did not raise serum cholesterol concentrations. Comparable cholesterol data are limited in the Δ^5-pregnenolone cases.

TABLE I

BODY WEIGHT, BLOOD AND SERUM DATA IN PATIENTS GIVEN Δ^5-PREGNENOLONE

Pt.		Interval*	Δ^5-Pregn. mg./day	Body Weight (kg.)	BLOOD Sugar mg.%	BLOOD NPN mg.%	SERUM HCO_3 mEq./L.	SERUM Cl mEq./L.	SERUM Na mEq./L.	SERUM K mEq./L.	SERUM Ca mg.%	SERUM P mg.%	SERUM H_2O gm./L.	SERUM CHOL. mg.%
M.M.		4/14	..	20.0	78	31	23.9	99.9	....	4.4	9.7	5.4	928	197
(Lupus 6½ F)	a.	4/14– 4/19	0	20.4	..	30	25.4	98.6	141.6	4.8	9.2	5.3	925	185
	b.	4/19– 4/24	1000	21.2	98	32	24.0	99.4	135.5	4.8	..	4.8	924	...
	c.	4/24– 4/29	1000	21.2	66	33	24.6	100.3	142.5	4.7	9.4	5.3	928	...
	d.	4/29– 5/4	1000	21.2	..	30	23.5	98.3	148.5	4.3	9.6	5.3	923	...
	e.	5/4 – 5/9	1000	21.4	..	35	25.1	98.6	....	..	8.7	5.4	920	...
	f.	5/9 – 5/14	0	21.6	76	34	24.5	100.9	140.0	5.8	9.2	5.9	927	...
	g.	5/14– 5/19	0	21.4	70	29	21.1	99.5	142.5	4.5	10.0	5.8	922	...
	h.	5/19– 5/24	0	21.8	115	31	23.4	98.6	138.5	4.2	9.0	5.6	924	...
	i.	5/24– 5/29	0	22.0	..	36	23.3	103.4	141.5	4.3	9.9	5.8	...	...
	j.	5/29– 6/3	0	22.0	88	31	22.7	102.1	140.0	4.4	9.5	6.2	924	...
L.G.		3/17	0	37.3	..	33	19.7	100.7	132.6	4.5	7.5	3.5	...	...
(Lupus 21 F)	a.	3/17– 3/19	400	...	77	31	20.5	103.8	134.8	4.5	..	3.9	946	203
	b.	3/19– 3/21	0	...	..	..	20.9	104.0	140.7	5.2	7.9	3.9	943	216
	c.	3/21– 3/23	0	...	83	45	20.8	104.6	137.1	6.6†	8.4	5.1	942	213
	d.	3/23– 3/26	235	35.6	..	43	21.2	102.1	131.0	5.0	7.5	4.0	941	230
	e.	3/26– 3/29	0	...	..	44	21.3	102.3	131.0	5.4	7.7	4.0	943	198
	f.	3/29– 4/1	500	34.8	..	..	21.1	103.3	140.7	5.1	8.3	4.3	943	178
	g.	4/1 – 4/7	0	34.6	..	31	21.2	100.2	133.5	4.5	9.0	4.0	945	...
M.F.		3/28	0	50.9	..	31	27.9	100.4	141.6	3.6	9.0	3.7	923	...
(Rh. Arthritis	a.	3/28– 4/2	500	50.7	..	30	28.3	105.3	146.9	4.3	8.9	4.1	931	...
27 F)	b.	4/2 – 4/10	0	50.6	..	28	27.9	99.0	152.9	4.5	9.0	4.5	935	229

D.H.	5/10	0	22.0	85	31	22.9	100.5	148.0	3.9	8.1	4.4	937	207
(Rh. Carditis	a. 5/10– 5/16	500 i.m.	22.6	81	34	23.3	102.0	142.5	4.2	8.9	5.7	926	...
6 M)	b. 5/16– 5/20	500 i.m.	22.0	76	36	23.2	103.8	149.0	3.8	9.4	5.2	...	215
	c. 5/20– 5/26	500 i.m.	22.6	76	35	25.3	102.2	144.0	4.6	10.1	5.4	930	...
	d. 5/26– 5/31	500 i.m.	21.8	57	40	17.0	98.1	137.0	3.9	10.4	4.8	929	...
	e. 5/31– 6/3	0	...	78	29	24.4	102.1	....	..	9.3	5.4	936	187
V.E.	11/13	0	12.8	84	..	23.3	101.6	140.5	4.3	..	..	921	...
(Rh. Arthritis	a. 11/13–11/24	1000 i.m.	12.4	82	32	20.8	91.1	136.0	4.1	9.7	4.2	...	159
7 F)	11/29	0	12.6	82	28	25.2	101.8	....	..	9.4	4.8	...	192
	b. 11/29–12/4	0	12.0	84	39	19.4	104.2	137.5	4.8	10.8	4.2	...	238
E.W.	5/17	0	39.7	70	48	24.7	99.1	140.7	4.2	..	..	...	...
(Rh. Carditis	a. 5/17– 5/25	500 i.m.	...	80	41	19.8	104.0	152.0	4.3	..	..	...	...
13 F)	b. 5/25– 5/30	1000 i.m.	...	66	36	24.7	96.5	138.5	4.6	..	5.0	...	122
J.M.	6/2	0	...	..	72	24.6	87.4	....	..	..	..	...	...
(Rh. Carditis	a. 6/2 – 6/6	740	25.9	100	34	27.0	82.3	142.0	5.4	..	..	933	...
8 F)													
F.W.	11/27	0	58.0	89	32	21.1	92.5	....	..	9.6	..	...	166
(Rh. Arthritis	a. 11/27–12/1	1000 i.m.	57.5	124	36	...	97.9	140.0	4.6	10.3	..	...	155
49 M)	b. 12/1 –12/12	0	57.7	89	34	25.5	99.7	140.5	5.1	10.4	5.2	...	154

* Samples drawn on date in second column.
† Pt. given KCl *per os* on 3/21.

TABLE II

BODY WEIGHT, BLOOD AND SERUM DATA IN PATIENTS GIVEN 21-ACETOXYPREGNENOLONE

Pt.	Interval*	21-Acetoxy Pregnenolone mg./day	Body Weight (kg.)	BLOOD Sugar mg.%	BLOOD NPN mg.%	SERUM HCO_3 mEq./L.	SERUM Cl mEq./L.	SERUM Na mEq./L.	SERUM K mEq./L.	SERUM Ca mg.%	SERUM P mg.%	SERUM H_2O gm./L.	SERUM CHOL. mg.%
W.McF.	11/18	0	...	88	44	22.7	104.3	139.0	4.2	9.8	4.2	927†	107
(Myel. Leuk.	a. 11/18–11/21	0	41.2	95	48	21.7	100.6	137.0	5.0	9.7	4.8	...	86
13 M)	b. 11/21–11/28	1000	38.8	91	50	22.2	98.6	132.0	3.8	..	4.0	...	114
C.V.	11/16	0	22.3	78	28	24.7	102.3	140.0	3.7	..	4.5	937†	225
(Lymph. Leuk.	a. 11/16–11/22	0	20.9	82	31	21.7	105.4	143.5	4.6	..	5.2	...	250
6 M)	b. 11/22–12/5	1000	19.8	75	29	24.4	99.6	136.0	4.8	..	5.0	935†	221
	c. 12/5 –12/12	1000	19.9	68	35	22.6	99.9	142.5	4.9	..	5.1	...	166
	d. 12/12–12/19	857	19.5	70	34	24.0	99.6	139.5	4.2	..	5.9	...	229
J.C.	11/22	0	49.8	86	34	23.2	98.7	143.5	4.4	10.2	4.6	...	202
(Rh. Arthritis	a. 11/22–11/28	1000§	49.4	75	36	21.8	99.6	140.0	4.3	10.3	4.6	...	195
14 M)	b. 11/28–12/2	0	48.6	73	35	22.4	101.8	142.0	4.4	10.3	4.7	...	194
D.S.	11/18	0	15.8	63	29	21.7	108.8	141.5	4.4	10.1	5.1	933†	241
(Rh. Arthritis	a. 11/18–11/21	0	14.6	68	37	...	107.6	130.0	4.7	10.6	5.9	...	205
3 F)	b. 11/21–11/28	1000§	14.2	77	33	23.0	98.7	137.5	..	9.9	..	...	...
F.W.‡	11/17	0	58.2	69	32	26.2	99.8	142.5	4.6	..	5.1	919†	127
(Rh. Arthritis	a. 11/17–11/22	1000	58.2	82	27	23.7	101.4	145.0	4.6	10.8	5.0	...	153
49 M)	b. 11/22–11/27	0	58.0	89	32	21.1	92.5	....	..	9.6	..	...	166
S.C.	11/22	0	...	75	75	22.6	80.6	125.0	5.6	8.1	3.9	...	149
(Rh. ht. disease	a. 11/22–11/24	0	83.3	91	62	24.6	76.0	116.5	5.1	8.9	3.6	...	149
42 M)	b. 11/24–12/1	1000	...	98	52	24.7	82.3	122.5	5.0	7.3	3.3	...	139
	c. 12/1 –12/4	1000	...	86	46	24.7	88.4	124.0	5.2	8.5	3.5	...	127
	d. 12/4 –12/12	0	75.0	78	31	26.1	94.2	133.5	4.6	8.9	4.1	...	120

* Samples drawn on date in second column.

† Calculated from serum total protein concentration.

‡ Subsequently received Δ^5-pregnenolone.

§ Administered orally during the last 2 days. All other pts. received the medication intramuscularly only.

C. External Exchanges of Chloride, Sodium, Potassium, and Nitrogen during Pregnenolone Therapy (Tables III and IV). The external balance data lend themselves to categorical description. Perhaps the most striking fact concerning the balance data as a whole is that trends evident in control periods prior to or well after pregnenolone therapy were not modified during the periods of drug administration. Thus, with but few exceptions the patients were in positive nitrogen balance prior to, during and after therapy with either steroid used. It is to be noted that the external balances of nitrogen have been corrected for changes in nonpro-

FIG. 5. L.G. Concomitant with and subsequent to Δ^5-pregnenolone therapy perceptible fading of the facial rash was observed without other clinical change.

tein nitrogen and therefore closely approximate in magnitude the balances of cell nitrogen as well. All but one of the patients on 21-acetoxypregnenolone were in positive potassium balance throughout observation and this was also true in most of those receiving Δ^5-pregnenolone. It is readily evident by inspection that in many instances a considerable proportion and at times all of the negative external balance of potassium, as in patient L.G., represented losses of cell potassium in conjunction with losses of cell nitrogen. The actual values can be estimated by using the ratio 1 gm. nitrogen = 2.4 mEq. potassium.[2, 3]

Insofar as external balances of sodium and chloride are concerned,

TABLE III

INTAKE, URINE VOLUME, TOTAL OUTPUT AND EXTERNAL BALANCE IN PATIENTS RECEIVING Δ^5-PREGNENOLONE

		INTAKE					OUTPUT*					EXTERNAL BALANCE			
Pt.	Interval	H_2O L.	Cl mEq.	Na mEq.	K mEq.	N gms.	Urine Vol. L.	Cl mEq.	Na mEq.	K mEq.	N gms.	Cl mEq.	Na mEq.	K mEq.	N† gms.
M.M.	a.	10.2	260	218	311	45.4	2.6	126	111	176	25.9	+134	+107	+135	+19.6
	b.‡	12.4	298	250	355	51.8	7.2	223	116	263	39.5	+ 75	+134	+ 92	+12.0
	c.‡	12.9	298	250	355	51.8	7.5	244	202	317	45.4	+ 54	+ 48	+ 38	+ 6.3
	d.‡	11.5	298	250	355	51.8	5.6	224	164	277	37.8	+ 74	+ 86	+ 78	+14.4
	e.‡	12.1	298	250	355	51.8	6.4	261	192	306	42.8	+ 37	+ 58	+ 49	+ 8.3
	f.	11.8	298	250	355	51.8	4.4	245	182	283	39.6	+ 53	+ 68	+ 72	+12.3
	g.	10.6	298	250	355	51.8	3.6	204	104	232	27.9	+ 94	+146	+123	+24.6
	h.	10.5	298	250	355	51.8	4.7	212	149	292	41.7	+ 86	+101	+ 63	+ 9.8
	i.	10.7	298	250	355	51.8	4.8	194§	134§	208§	34.6§	+104	+116	+147	+16.5
	j.	10.3	298	250	355	51.8	4.0	215	156	233	35.2	+ 83	+ 94	+122	+17.3
L.G.	d.‡	2.8	55	46	65	9.5	1.6	53§	20§	112§	13.5§	+ 2	+ 26	− 47	− 3.5
	e.	2.6	36	30	43	6.2	1.4	20	11	107	15.9	+ 16	+ 19	− 64	− 9.9
	f.‡	3.5	74	62	89	13.0	2.2	24	12	142	23.5	+ 50	+ 50	− 53	−10.5
M.F.	a.‡	12.5	273	229	326	47.6	7.4	259	177	597	43.8	+ 14	+ 52	−271	+ 4.1
	b.	15.3	438	368	524	76.4	10.3	375	368	589	72.2	+ 63	0	− 65	+ 4.9
D.H.¶	a.‡	13.2	363	300	426	61.0	4.7	280	271	187	53.8	+ 83	+ 29	+239	+ 6.8
	b.‡	10.0	242	200	284	40.7	6.0	207	159	358	57.0	+ 35	+ 41	− 74	−16.6
	c.‡	12.5	352	291	414	59.2	5.0	272§	201§	266§	49.3§	+ 80	+ 90	+148	+10.0
	d.‡	9.3	240	199	282	40.4	3.6	187	158	236	32.9	+ 53	+ 41	+ 46	+ 6.8
	e.	7.4	197	162	230	33.0	1.7	81§	55	94	20.0	+116	+107	+136	+11.4
V.E.	a.‡	18.8	243	14	590	107.7	3.0	95	50	188	50.3	+148	− 36	+402	+57.5
	b.	6.2	81	5	196	35.8	1.3	41	15	115	25.9	+ 40	− 10	+ 81	+ 9.0
E.W.	b.‡	10.1	132	8	321	37.8	8.6	448	4	284	69.3	−316	+ 4	+ 37	−30.2
F.W.	a.‡	10.5	150	124	176	39.4	6.2	224	128	276	45.4	− 74	− 4	−100	− 7.5
	b.	28.3	518	428	608	136.4	21.0	386	403	656	143.7	+132	+ 25	− 48	− 6.5

* Includes amounts excreted in urine, stool, vomitus and removed in blood.

† Corrected for changes in NPN ($N\dagger$ = External balance of $N - \frac{(0.65 \text{ B. wgt.} \times \Delta \text{ NPN})}{1000}$)

‡ Δ^5-pregnenolone period.

§ Output does not include stool. With the exception of K the absence of stool data affects external balances only minimally.

¶ Also received cation exchange resin during period b.

TABLE IV

INTAKE, URINE VOLUME, TOTAL OUTPUT AND EXTERNAL BALANCE IN PATIENTS RECEIVING 21-ACETOXYPREGNENOLONE

		INTAKE					OUTPUT*					EXTERNAL BALANCE			
Pt.	*Interval*	*H_2O L.*	*Cl mEq.*	*Na mEq.*	*K mEq.*	*N gms.*	*Urine Vol. L.*	*Cl mEq.*	*Na mEq.*	*K mEq.*	*N gms.*	*Cl mEq.*	*Na mEq.*	*K mEq.*	*N† gms.*
W.McF.	b.‡	17.4	263	105	210	96.8	7.9	192§	94§	389§	128.2§	+ 71	+ 11	−179	−31.6
C.V.		1.8	25	2	61	11.1	0.6	12	21	19	7.4	+ 13	− 19	+ 42	+ 3.7
	a.	11.0	151	9	366	66.7	2.2	60	56	75	17.1	+ 91	− 47	+291	+49.2
	b.‡	23.0	356	21	863	157.6	6.9	108	18	221	65.0	+248	+ 3	+642	+92.9
	c.‡	12.8	204	12	496	90.6	3.5	49	7	142	33.5	+155	+ 5	+354	+56.3
	d.‡	13.4	229	14	554	101.3	3.6	114	33	156	37.0	+115	− 19	+398	+64.3
J.C.	a.‡	12.0	160	10	387	70.7	2.4	104	28	206	59.0	+ 56	− 18	+181	+11.1
	b.	8.1	78	5	190	34.7	1.4	4	8	109	31.3	+ 74	− 3	+ 81	+ 3.7
D.S.	a.	6.3	106	6	258	47.2	1.3	51	41	78	17.7	+ 55	− 35	+180	+28.8
	b.‡	11.8	163	10	395	72.1	3.5	91§	27	209	45.8§	+ 72	− 17	+186	+26.7
F.W.¶	a.‡	10.7	204	169	240	53.9	9.6	336	..	186	...	−132	...	+ 54	...
	b.	14.7	262	216	308	69.0	9.0	170	75	285	...	+ 92	+141	+ 23	...
S.C.	a.‡	20.4	223	13	540	98.6	5.5	4	9	237	60.4	+219	+ 4	+303	+43.6
	b.‡	6.9	92	6	224	40.9	3.8	4	6	154	41.9	+ 88	0	+ 70	+ 2.2
	c.	20.6	240	14	581	106.2	16.8	197	22	377	121.6	+ 43	− 8	+204	− 8.1
	d.	7.5	44	3	106	19.3	5.1	87	6	42	25.3	− 43	− 3	+ 64	− 5.0

* Includes amounts excreted in urine, stool, vomitus and blood drawn for analyses.

† Corrected for changes in NPN.

‡ Indicates 21-acetoxypregnenolone period.

§ Does not include stool.

¶ Subsequently given Δ^5-pregnenolone.

it is readily evident that the adult subjects treated with either steroid tended to be in equilibrium with minor degrees of sodium and chloride retention interspersed among negative balances. In the children, however, especially in M.M. and D.H. in Table III, sodium and chloride were retained. Since this occurred to an equal extent in the pregnenolone and nonpregnenolone periods, it cannot be ascribed directly to the drug. It may be more than chance however that these two patients experienced a convincing remission in their disease and were gaining weight and presumably growing and replenishing their stores of these and other body constituents.

FIG. 6. V.E. Intramuscular administration of Δ^5-pregnenolone, 1.0 gram per day, resulted in pain and swelling at the injection sites, fever, and a greater leukocytosis with PMN increase. These all disappeared following withdrawal of the drug. The sedimentation rate was not affected, nor did the patient's clinical status change. The absolute eosinophil count did not drop.

D. Absence of Pregnenolone and 21-Acetoxypregnenolone Effect Upon Indices Usually Altered by 11-Oxygenated Steroids (Tables V and VI). The steroids in general did not produce relative or absolute eosinopenia nor did the uric acid-creatinine ratio rise with any degree of regularity. 17-Ketosteroid output was variable but the fluctuations were small and without definable trend. Finally it is clear from the recorded protein-bound iodine values that pregnenolone therapy did not lower this iodine fraction.

Discussion

Evaluation of the clinical effects of these two steroids must certainly be tentative and preliminary in view of the limited number of patients in any particular disease category in this series. Another important obstacle is readily evident upon perusal of Fig. 4. This patient was apparently heading toward at least a partial spontaneous remission at the time pregnenolone therapy was started. Other limitations in our studies include the

FIG. 7. S.C. This 42-year-old male with a history of previous rheumatic heart disease was admitted in severe congestive failure and received 1 gm. of 21-acetoxypregnenolone daily for 10 days without local reaction. Improvement during therapy which also included Na restriction, digitalis, and bed rest was gradual but still incomplete at the end of 5 weeks.

variability in dosage schedules and in route of administration, often imposed by clinical exigencies. On the other hand there is suggestive evidence that in several of the patients (Figs. 1, 5, 7, 8) the clinical course was favorably affected by these non-11-oxygenated steroid compounds, Finally it is clear that in the remainder the illness continued unabated.

These comments on therapeutic efficacy logically evoke questions concerning possible mode or modes of action. Pregnenolone action may be direct on tissues or may affect cell activity via metabolic pathways not detectable by the methods employed in our studies. The absence of any

TABLE V

PROTEIN-BOUND IODINE, BLOOD EOSINOPHILS AND URINARY 17-KETOSTEROIDS, URIC ACID, AND CREATININE IN Δ^5-PREGNENOLONE THERAPY

			BLOOD		URINE		SERUM
Pt.	*Interval*	*Δ^5-Pregn. mg./day*	*EOS/mm.³*	*17-Ketosteroids mg./day*	*Uric Acid mg./day*	*Creatinine mg./day*	*Protein-Bound Iodine µg.*
M.M.	4/14– 4/19	0	125	1.6	125	200	..
	4/19– 4/24	1000	125	4.2	107	188	..
	4/24– 4/29	1000	88	4.6	225	158	5.1
	4/29– 5/4	1000	125	3.6	60	45	6.3
	5/4 – 5/9	1000	50	3.5	237	323	..
	5/9 – 5/14	0	25	0.8	154	243	5.5
	5/14– 5/19	0	94	0.8	252	245	4.1
	5/19– 5/24	0	119	1.1	156	308	..
	5/24– 5/29	0	213	3.4	174	...	6.8
	5/29– 6/3	0	194	3.6	211	...	..
L.G.	3/17	0	0	..	...	...	4.4
	3/19– 3/21	0	...	1.4	...	229	..
	3/23– 3/26	235	...	1.0	117	563	4.6
	3/26– 3/29	0	...	1.0	45	442	..
	3/29– 4/1	500	...	2.1	50	633	..
M.F.	3/28	0	...	..	...	...	4.3
	3/28– 4/2	500	...	12.8	72	892	..
	4/2 – 4/10	0	...	8.0	85	852	4.3
D.H.	5/10	0	369	..	...	...	3.1
	5/10– 5/16	500 i.m.	256	1.6	516	62	..
	5/16– 5/20	500 i.m.	363	1.6	452	409	..
	5/20– 5/26	500 i.m.	475	1.6	107	60	..
	5/26– 5/31	500 i.m.	275	1.7	173	73	3.8
	5/31– 6/3	0	840	2.1	256	248	..
V.E.	11/13–11/24	1000 i.m.	81	..	268	124	4.7
	11/29	0	118	..	...	...	..
	11/29–12/4	0	369	..	102	110	4.2
E.W.	5/16– 5/17	0	106	1.6	396	...	..
	5/24– 5/25	500 i.m.	81	1.8	584	437	..
	5/25– 5/30	1000 i.m.	119	4.8	716	604	..
J.M.	6/3 – 6/4	740 i.m.	19	..	781	...	..
	6/6	740 i.m.	6	..	...	...	..
F.W.	11/22–11/27	0	185	..	351	940	..
	11/27–12/1	1000 i.m.	963	..	315	886	..
	12/1 –12/12	0	144	..	372	706	..

incontrovertible and consistent influence of either Δ^5-pregnenolone or 21-acetoxypregnenolone on external transfers of nitrogen and electrolytes in this series of patients has already been emphasized in the results. It could be, however, that the observed effects are related to a conversion of the administered material to derivatives which are physiologically active, perhaps to 11-oxygenated steroids. There is experimental support for

TABLE VI

PROTEIN-BOUND IODINE, BLOOD EOSINOPHILS AND URINARY 17-KETOSTEROIDS, URIC ACID, AND CREATININE IN 21-ACETOXYPREGNENOLONE THERAPY

			BLOOD	*URINE*			*SERUM*
Pt.	*Interval*	*21-Acetoxy Preg-nenolone mg./day*	*EOS/mm.³*	*17-Keto-steroids mg./day*	*Uric Acid mg./day*	*Creati-nine mg./day*	*Protein-bound Iodine µg.*
W.McF.	11/16–11/17	0	5088	..	437	...	4.7
	11/21–11/28	1000	2375	..	583	1031	3.9
C.V.	11/16–11/17	0	0	..	472	293	..
	11/17–11/22	0	0	..	137	136	3.2
	11/22–12/5	1000	19	..	144	194	..
	12/5 –12/12	1000	0	..	123	171	3.7
	12/12–12/19	857	3	..	217	199	..
J.C.	11/22	0	206	..	...	...	4.2
	11/22–11/28	1000	75	6.9	69	554	..
	11/28–12/2	0	263	3.4	49	486	6.0
D.S.	11/18	0	144	..	...	...	5.5
	11/18–11/21	0	225	..	37	191	..
	11/21–11/28	1000	200	..	177	217	..
F.W.	11/17	0	88	..	...	...	4.0
	11/17–11/22	1000	6	..	307	1002	4.4
	11/22–11/27	0	185	..	351	940	4.8
S.C.	11/24	0	44	..	...	...	2.0
	11/24–12/1	1000	113	..	88	456	2.5
	12/1 –12/4	1000	131	..	203	684	2.6
	12/4 –12/12	0	244	..	352	743	2.7
	12/12–12/15	0	544	..	366	...	3.0

such a process in the demonstration that perfusion of isolated adrenals with Δ^5-pregnenolone increases, though not in equivalent amounts, the output of Compound F.[9] It has already been pointed out, however, that the repeated daily administration of as much as 1000 milligrams of either pregnenolone, *per os* or parenterally, failed in our studies to produce changes in indices ordinarily altered by smaller amounts of Compound E, ACTH, or both, *i.e.*, in serum electrolyte values, blood sugar levels, circulating eosinophils, uric acid-creatinine excretion, or the 17-ketosteroid output.[7, 10] In addition evidence has been presented indicating that the

pregnenolones did not depress the circulating protein-bound iodine nor did they raise the serum cholesterol in the manner characteristic of the 11-oxygenated steroids or of ACTH.[11, 12] Hence if an *in vivo* conversion represents a metabolic fate and the mode of beneficial action of pregnenolones, these compounds should be given in amounts considerably in excess of the usual dosage of 11-oxygenated steroids, and perhaps even in excess of amounts used in our studies. Prolonged therapy is obviously desirable, since it may be that with time the efficiency of the conversion process rises.

FIG. 8. C.V. Intramuscular 21-acetoxypregnenolone was administered for 31 days to this boy with acute lymphatic leukemia with only minor local reactions. Fever had been present prior to therapy. During the period of pregnenolone administration, either coincidentally or as a therapeutic response, the PMN increased, lymphocytes decreased, and the erythrocytes and hemoglobin either did not fall further or actually rose slightly.

Some comment is also necessary concerning routes of pregnenolone administration. Particular care must be taken to keep in mind the possible effects that local reactions or sterile abscesses induced by parenterally injected pregnenolone may have on the course of illnesses. This complication renders uncertain whether or not pregnenolones are being absorbed. It may also serve as a stimulus to increased endogenous adrenal cortical activity and thus ameliorate the disease state, or it can on the

other hand mask or cancel improvement induced by pregnenolone therapy.

Oral administration which in our experience is unassociated with any detectable toxic reaction obviates this complication but leaves unsettled the question of completeness of absorption. Perhaps the chief justification for use of this route is the demonstration in our laboratory[13] that Compound E produces the characteristic eosinopenia when given either *per os* or parenterally.

FIG. 9. W.McF. The progressive downhill course of this patient with acute myelogenous leukemia was not retarded by repeated transfusions or by parenteral treatment with 21-acetoxypregnenolone. The administration of the drug produced local swelling and tenderness associated with fever and increased leukocytosis which diminished when therapy *per os* was substituted for the parenteral route.

It is obvious that with the data herein presented we have only begun to explore the clinical potentialities of pregnenolones. These studies have served, however, to stimulate our interest concerning procedures or experimental approaches which might prove informative in further studies. It is clear first of all that the parenteral forms of these compounds need to be modified to obviate local reactions and to assure complete absorption. Then it may well be advantageous to continue to attempt to equate the pregnenolones with the 11-oxygenated steroids. This could not only indicate whether the former, perhaps themselves inactive, merely provide material for greater endogenous production of other steroids but also

clarify any quantitative aspects of this process. If such an interconversion does exist it may be possible to potentiate the process by concomitant ACTH therapy, by complete sodium restriction, or both. Finally, in view of the evident low toxicity of pregnenolones the desirability of long-continued administration of these and related compounds in large dosage in therapeutic trials seems justified. These approaches may unearth less toxic or less expensive therapeutic agents, or ones whose effects are mediated via mechanisms unrelated to the adrenal cortex or to 11-oxygenated steroids.

FIG. 10. D.S. Intramuscular injection of 21-acetoxypregnenolone produced local reactions, hyperpyrexia, leukocytosis with PMN increase, and an increase in the sedimentation rate without clinical improvement. The eosinophils did not drop. Subsequent administration of 21-acetoxypregnenolone at a time when the patient was receiving 16-dehydropregnenolone did not raise the temperature, suggesting that the earlier reaction to 21-acetoxy did not represent drug fever.

Summary and Conclusions

1. Δ^5-Pregnenolone or 21-acetoxypregnenolone has been administered parenterally or *per os* to 3 patients with active rheumatic fever with myocarditis, 2 with disseminated lupus, 2 with acute leukemia, 5 with active rheumatoid arthritis, and 1 with chronic rheumatic heart disease and severe congestive failure.

2. Despite the large dosage employed (up to 1000 mg. per day for periods up to 3 weeks) these compounds appeared to be nontoxic. Local

reactions often associated with fever, leukocytosis and, in two instances, sterile abscess formation did occur at times with the parenteral forms. There was no evidence during therapy of any alteration in whole blood NPN, fasting blood sugar levels, serum CO_2, Cl, Na, K, Ca, P, or H_2O concentrations. External balances of Cl, Na, K, and N were not discernibly influenced. Neither did the compounds produce any patterns of change in the circulating eosinophils, in the urinary 17-ketosteroids, uric acid, and creatinine, nor in the serum levels of cholesterol or protein-bound iodine.

3. Clinical improvement was noted in 2 of the 3 patients with rheumatic fever, in the 2 cases of lupus, and, possibly, in 1 of the 2 leukemia patients as well as the 1 subject with congestive failure.

4. The precise relationship between these clinical results and pregnenolone therapy needs to be investigated by further study. Our data suggest that these clinical results were unassociated with effects ordinarily present during 11-oxygenated steroid activity.

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Discussion of Chapters 18, 19, and 20

J. Flores Espinosa: I have been interested in the treatment of rheumatoid arthritis for the past 14 years and I am very much impressed by how difficult it is to evaluate the results of the different treatments, as has been pointed out in the papers presented yesterday and this morning. Actually, in the 14 years that I have been treating rheumatoid arthritics I have never found as satisfactory results as those obtained with cortisone and other steroids of the type of pregnenolone. For these reasons I think it is possible to evaluate our results if we reach an agreement as to our interpretations. Until now there have been great disagreements, as Dr. Conn pointed out last evening, as to what is the meaning of "remission." Also, what do we mean by "improvement"? To some it may mean only a slight improvement, to others it is interpreted as a marked improvement, and we have not reached any agreement as to the meaning of these terms. This is of enormous importance to me because I want to tell you about the experience we have had at the Hospital General of Mexico in the treatment of rheumatoid arthritis, using pregnenolone in doses relatively lower than those used by our North American colleagues. From this conference some useful conclusions should be reached and general recommendations made in order to have a uniform concept of the following points.

First, as to diagnosis, the need of recognizing the different degrees of rheumatoid arthritis, and of separating them from other types of rheumatic diseases. I have had the opportunity to see how neuritis, polyneuritis, and other diseases due to defective posture have been mistaken for rheumatoid arthritis. In the former cases the use of an anti-rheumatic drug does not have comparable effects to those obtained in the latter condition. Therefore, I believe it is indispensable to make an exact diagnosis by means of precise radiologic and laboratory methods in addition to the pertinent clinical information, so that there will be no confusion in differentiating these diseases.

Second, there is the need for uniform criteria by which to judge the effec-

tive improvement of the patients with respect to general reactions, such as Dr. McGavack has mentioned, as well as to evaluate local manifestations, both articular and periarticular, and the psychological condition of the patient. It is also necessary that there be developed a means of obtaining uniformity in the doses of steroids used. The apparently contradictory results revealed in the different papers presented yesterday and this morning are, in my opinion, due to the use of different materials by diverse routes of administration. The parenteral administration of pregnenolone in aqueous suspension is so painful that we had to discontinue its use; it has been used by mouth in doses varying from 100 up to 900 mg. daily by different investigators, and still others have used the oil solution by intramuscular injection, which is much better tolerated than the aqueous suspension. This means then that in order to be able to compare the studies made in different institutions and in different countries, it is absolutely necessary that the investigators follow a uniform system as to the method of administration, the type of drug, the dose to be used, and the duration of the treatment. Otherwise, we will find such negative results as those of Dr. Robles Gil when he alternates ACTH or cortisone with pregnenolone, which steroid he used only for 10 days in doses of 300 mg. by mouth or 100 mg. by intramuscular injection. His results are very different from those obtained with more prolonged administration and higher dosage, as has been done by our North American colleagues, who do find subjective and objective improvement in a variable percentage of rheumatoid arthritics even when pregnenolone is given in association with testosterone, for example, as was presented in Dr. Hellbaum's paper yesterday. It is, therefore, absolutely necessary that this conference plan a definite orientation for the future, so as not to continue in the present state of anarchy regarding the results, which are classified as wonderful by some, mediocre by others, and nil by still others, but with investigators using different methods as to diagnosis, evaluation of improvement, method of administration, dosage, and duration of the treatment. If we want to obtain a correct idea of the value of steroids in the treatment of rheumatoid arthritis, we should study a large series of patients for long periods of time and with uniform methods which will permit a comparison of results obtained, whether the work was done in Canada, the United States, the Argentine Republic, Colombia, Puerto Rico, or Mexico City. If we do not follow this system we will continue to find that Dr. McGavack is optimistic from his results with pregnenolone, while Dr. Freyberg is pessimistic in relation to his, which means that the methods employed have been different. Dr. Smith finds improvement in his cases of fibrositis, while other investigators will not even accept that fibrositis be included in these studies. We are talking different languages in different ways. If we want to understand each other, we must standardize our future work, and this, I believe, will be the greatest achievement of this conference.

As far as I am concerned, I have obtained favorable results in rheumatoid arthritis using pregnenolone in oil solution by intramuscular

injection in doses as low as 100 mg. daily. This dose level compares favorably with previous methods of treatment, such as the use of gold salts, high doses of vitamin D, x-ray therapy, fever therapy, and other methods. I don't mean by this that I am totally optimistic and that I believe that pregnenolone, ACTH, or cortisone will cure rheumatoid diseases. Far from it; I simply believe that up to now it has been demonstrated that there are reversible changes in patients previously considered incurable and to have irreversible changes. If we have demonstrated this reversibility, it is possible to find a way to obtain better substances and better treatments that may effect a definite cure in so terrible a disease as rheumatoid arthritis.

E. Arce Gomez: With respect to the use of pregnenolone in rheumatoid arthritis, our series of treated cases is relatively small, but the data are definite. A daily dose of 450 mg. intramuscularly has produced the best results. Also, in other pathological states such as bronchial asthma, a dose of 400 mg. of pregnenolone daily has produced an increase of 400 to 500 cc. in vital capacity in four of five treated cases.

C. Ascoitia: I have done some experimental work designed to study the rate of absorption of pregnenolone and of the 21-acetoxypregnenolone in microcrystalline suspensions, as well as the rate of absorption of esters of pregnenolone in oil solution. The method used was as follows. A dose of 100 mg. at a time was given in a single injection to rabbits; the animals were sacrificed at different periods, and the steroid recovered by extraction of the tissue at the site of injection. The product was purified and the recovered steroid weighed. When we worked with pregnenolone esters in oil solution, it was impossible to recover the ester form, but free pregnenolone was recovered. From this one could calculate the equivalent of the injected ester which was recovered. It was found that 24 hours after injection of pregnenolone in microcrystalline suspension, it was possible to recover about 79.9 per cent of the material injected; 7 days after the single injection it was possible to recover 58.7 per cent; and after 15 days, 44.3 per cent remained at the site of injection. When we injected still smaller crystals (2 to 3 μ), the speed of absorption was not increased. When we used the pregnenolone esters in oil solution, it was possible to find only traces of the steroid 48 hours after the injection.

R. T. Smith: During our 10-minute recess just ended, I spoke to Dr. McGavack and asked him a question. I think I can save time by telling you what he said. If you remember in his discussion, he mentioned the fact that in patients with osteoarthritis, the group with periarticular involvement showed the greatest improvement after pregnenolone therapy. I asked whether the rheumatoid arthritic patients showed much the same sort of thing and Dr. McGavack replied in the affirmative. That falls into line with what we were presenting yesterday morning. In other words, in breaking down the symptoms and findings as he did, Dr. McGavack

classified the fibrositic elements into the periarticular group. He kept the local rheumatoid elements in the bone and joint group, designated as a systemic group and a psychogenic group, much as we did with the diagrams we showed yesterday morning. I think that this fits into the idea that the greatest improvement in any case of rheumatoid arthritis or any rheumatic disease is really improvement in the fibrositic part of the disease. In osteoarthritis, particularly, things are not always as they seem. Unfortunately there are too many instances in the literature of patients designated as osteoarthritic who are really patients suffering from fibrositis, with incidental evidence by x-ray examination that they are growing old. We know that from time to time when we treat people with osteoarthritis, they improve despite the fact that bony changes continue to advance. The cartilage gradually wears away and there is no rhyme or reason why improvement should occur while the bone changes worsen, if the symptoms are actually due to the osteoarthritic changes.

Some years ago Dr. Bauer and his co-workers did a fine study of normal knee joints. If one goes through that study he will find illustrations of joints from individuals of all ages who had no symptoms referable to the knee joint, yet they show progressive aging changes beginning shortly after the joints mature. Within 5, 10, or 15 years after the joints matured, there were aging changes that could be seen with x-ray whereas earlier, in the first few years after the joints matured, there were microscopic changes. All of us are going to grow old unless we get in the way of an automobile or shorten our lives by illness, and all of us will develop these wear and tear changes. The difference in symptomatic or asymptomatic osteoarthritis is simply whether the cartilage of the joint has eroded so rapidly that compensatory changes cannot keep pace or whether we go on at our usual rate of speed, with daily micro trauma gradually wearing away the cartilage at a rate permitting the body time to compensate for it by thickening the subchondral plate, etc. When we treat people who have x-ray evidence of osteoarthritis but who have purely fibrositic symptoms and give them rehabilitative exercises to make their muscle function better, they lose their symptoms. I think that this accounts for reports of improved osteoarthritic patients. They are incorrectly labeled.

I don't remember that Dr. McGavack said anything about improvement in swelling. I suspect he did not comment on this because there was no definite improvement in swelling or in the true inflammatory elements of the rheumatoid arthritis. This has been our experience.

Some of the other collagen diseases were mentioned as having been improved by pregnenolone. We have an instance of a 10-year-old boy who had evidence of dermatomyositis, established by biopsy. He was given ACTH for a period of about three weeks with little or no change in his rash or in his joint symptoms. He was then changed to cortisone and continued on it for a little over three weeks at which time it was stopped because he was developing a buffalo hump, rounded facial characteristics, and some of the other toxic symptoms. He had no therapy for a period of a week. He still had his rash. He was then placed on pregnenolone—

200 mg. three times daily—and para-aminobenzoic acid—2 gm. four times daily. After one month, the rash had completely disappeared and he had no pain in his knees, which were the only joints involved. He had gained weight. He still had some fatigue, but he was able to go up and downstairs without dragging himself by pulling on the bannister.

Two adult patients with scleroderma were treated first with para-aminobenzoic acid, giving each 12 gm. a day and gradually increasing to 14 gm. in one and 16 gm. in the other. Both of the patients had advanced changes of the hand and face and one had involvement of the esophagus. In each instance there resulted a loosening of the skin. The skin became looser on the hands, and the face and forehead could be wrinkled. The skin over the dorsum of the hand and over the proximal phalanx of all of the fingers except the fifth fingers could be picked up with the fingers after two months of treatment. We finally gave these patients, along with the para-aminobenzoic acid, 600 mg. of pregnenolone daily by mouth. The previous improvement, which had apparently become static over a period of a month following the para-aminobenzoic acid alone, has progressed until the striae in the skin of the fingers can be seen. There is loosening of the skin over the middle phalanges with some reappearance of the striae in the skin of the terminal phalanges although the skin is still tight over the terminal phalanges. The one patient had received pregnenolone alone in doses of 600 mg. a day previously, about six months before she was placed on the para-aminobenzoic acid. During the period of six weeks that she received this drug she thought she felt better although there were no demonstrable signs. But now, with this combined therapy she has been coming along much more rapidly than while on the para-aminobenzoic acid alone. She had some discomfort in the past on swallowing but this has disappeared recently. This would suggest that there may be some benefit in the combination of these two drugs in scleroderma and dermatomyositis.

T. H. McGavack: The improvement in the fibrositic manifestations is the only important effect of pregnenolone in osteoarthritis. Often the relief of the periarticular pains brings into prominence an awareness of the pain on motion of the joints. This is the only condition in which the action of pregnenolone closely parallels that of ACTH and cortisone.

E. B. Astwood: In seeking an explanation for the widely divergent reports on the effectiveness of pregnenolone, it has occurred to me that two considerations might resolve the difficulty. In the first place, it is clear from the papers that have been presented that widely different criteria of improvement have been used. For example, Dr. McGavack has reported a 50 per cent incidence of improvement in a variety of diseases and has, therefore, classified the compound as 60 per cent as effective as ACTH. At the other extreme, others have cited entirely negative results. Dr. Freyberg made the very interesting observation that if more than 400 mg. a day were given, some slight improvement would occur in

52 per cent of cases, but concluded that real objective improvement was rare, in agreement with the findings of Dr. Bauer and others. In the second place, a few examples of striking improvement have been well documented. These have been associated with clearcut objective criteria of improvement, such as a fall in the sedimentation rate. Now, it has already been shown that pregnenolone does not change the sedimentation rate, so when the sedimentation rate does fall, it suggests that some other

Fig. 1. Effect of pregnenolone on rheumatoid spondylitis.

factor such as spontaneous remission is operative, as in the case of disseminated lupus erythematosus reported by Dr. Danowski this morning and in the case of rheumatoid arthritis that I referred to yesterday.

S. H. Bassett: I would like to show three slides on a single case of rheumatoid arthritis and spondylitis. This case is that of a young man of 25 who has had the disease for approximately six years and who was in essentially an inactive phase when these studies were done. *Fig. 1* shows some of the clinical findings; the patient's weight is recorded at the top; the temperature, as you see, is normal; the pulse rate is normal, the systolic and diastolic blood pressures do not change. At the point indicated in the figure, the patient was given 2 gm. of pregnenolone acetate orally

for 20 days and I think the only change that appears in this figure that may possibly be of some significance is a very slight decline in the rise in weight which this patient was showing throughout the course of the study. Then, after 14 days of a control period, the patient was given 400 mg. of an aqueous suspension of pregnenolone acetate intramuscularly without abscess formation (in this case) and without rise in fever. He did have pain at the site of injection and some induration. Again I think there is possibly a little flattening of the weight curve when the medication was given.

Fig. 2 shows some of the findings in the serum, the concentration of serum sodium, chloride, and potassium, and the eosinophil counts. I think you will agree that there are essentially no changes throughout so far as these factors are concerned. Everything is normal and remains normal throughout the course of the study.

Fig. 2. Effect of pregnenolone on rheumatoid spondylitis.

In *Fig. 3* we have charted the external balances of nitrogen, potassium, sodium, and chloride; nitrogen at the top, followed by potassium, sodium, and chloride in order. Fecal excretion is represented by the hatched area at bottom; urinary excretion is charted above it; the average nitrogen intake is given as a straight line at 16 gm. This patient was on a constant weighed diet throughout the entire time. The diet was analyzed for the various constituents mentioned and everything as far as possible was checked constantly throughout the entire course of the study. There is possibly a little rise in urinary nitrogen excretion during the time when the patient first received the pregnenolone, and no apparent increase during the second period when he received it intramuscularly. I would say that the potassium balance is likewise essentially one of equilibrium. For the entire period of the study the patient did have a slight positive

nitrogen balance which would have accounted quite adequately for his increase in weight. He gained approximately 1.5 kg. in 65 days. The potassium balance is too positive for the nitrogen balance and this may possibly be in error. I think there is nothing with respect to the sodium and chloride balance in this patient which is significant. As you see, there is considerable fluctuation from day to day but the overall picture is essentially what one might expect, that is, moderate retention of both sodium and chloride. The reason for this is that we have no accurate way

Fig. 3. Effect of pregnenolone on rheumatoid spondylitis. Metabolic balance data.

of measuring the loss of electrolytes from the skin. Our studies were done in Southern California in a fairly warm climate where moderate sweating is to be expected. We find that most of our patients do show positive balances of sodium chloride. If there had been any marked change due to the administration of pregnenolone in either of the experimental periods, I would have expected it to have become evident either during the time the agent was administered or in the after period. (When cortisone or ACTH are given, the effect on electrolytes may become apparent only in the after periods.) So far as I can see, the data confirm the results of Dr. Danowski, at least in this single patient.

J. W. Conn: I should like to make a comment on the metabolic aspects of pregnenolone. From what has been reported previously, and from what has been reported at this meeting as well, I think that there is very little evidence that pregnenolone exerts a metabolic effect in any of the spheres that have been studied to date. This brings up again a point that I made yesterday, and on which I'd like to enlarge. We said yesterday that there is no metabolic change that has yet been measured that always parallels the improvement of the arthritic patient given ACTH or cortisone. Nor is there any change in the metabolic indices that have been followed to date, which is different in the arthritic patient from that observed in normal people given these compounds. The same statement applies as well in connection with diseases other than arthritis which respond well to ACTH or cortisone. The point that I would like to make is that after the administration of a substance, the absence of metabolic changes of the type that we have become stereotyped into studying does not necessarily mean that there is not an antirheumatic property to that substance. Obviously, when a patient with rheumatoid arthritis improves, there have been metabolic changes. We simply have not learned to measure what those metabolic changes are. One point that should be made in this connection is that the few experiments that have been made with Compound F, 17-hydroxycorticosterone, have shown great antirheumatic effects, but essentially no metabolic effects in man. This is surprising in view of reported animal work with Compound F. Obviously, again, with Compound F there are metabolic effects going on as indicated by relief of arthritis. I would like to call on Dr. Sprague to comment since he has had some personal experience with the metabolic effects of Compound F in man. Perhaps Dr. Dobriner, who also knows some of the effects of Compound F in man, would also like to comment.

R. G. Sprague: We hope to have more Compound F in the future for further study of its antirheumatic and physiologic properties. It would surprise me if it proved to have less metabolic activity than cortisone. It is pertinent to recall that several years ago we reported a case of Cushing's syndrome associated with diabetes and alkalosis and the excretion of Compound F in the urine. Presumably Compound F, produced by hyperplastic adrenal cortices, was largely responsible for the metabolic and clinical features of Cushing's syndrome in this case.

K. Dobriner: The metabolic actions and chemical effects of the steroids which have been discussed are dependent upon the amounts which circulate and only indirectly on the amounts administered. It should be kept clearly in mind that these compounds are for the most part extremely insoluble substances; many of them, in addition, form hard dense crystals. We know very little about their absorption from the site of injection or whether they are absorbed from the intestinal tract. These facts pose a fundamental problem, the solution of which would enable us to assess more accurately the conflicting evidence which has been re-

ported here. This information is badly needed. It should and could be provided most easily by studies with labeled compounds. In our Institute (with Drs. Gallagher, Fukushima, Rosenfeld, and Hollander) we found that labeled cholesterol in a fine suspension in saline was, for all practical purposes, not absorbed following intramuscular injection. Other steroids, e.g. testosterone, were rapidly absorbed, metabolized, and excreted. Unless the factor of absorption is known and controlled, much of the clinical evaluation of a substance is of dubious significance.

The problem of an effective dose of, for example, progesterone, might be estimated from the urinary excretion of pregnanediol during pregnancy, and I have discussed this in my previous presentation. Granting that my estimates are only approximations, there can be little question that the amount required of some compounds, at least, is very large. It is, therefore, not unreasonable to suggest that the seemingly large amounts employed in many studies may well be in the physiologic range.

J. W. Conn: Inasmuch as one of the central themes is the need for something with which to measure antirheumatic effects besides clinical observation, I wonder if Dr. Holbrook would summarize very briefly his studies of amino acid accretion?

W. P. Holbrook: I was not expecting to talk about this, but I'll summarize, as Dr. Conn has suggested, very briefly. We've been interested in amino acid metabolism in rheumatoid arthritis for several years and I may say that the idea which started this study on amino acids was the evidence of changes in the histidine excretion in pregnancies where remissions in the arthritis occurred. We began studying some 17 of the amino acids in blood and urine. When I speak of the histidine pattern, it involves two or three of the other amino acids in less magnitude. We found that in rheumatoid arthritis there is consistently a lowered histidine excretion and a near normal blood level. When remission occurs, whether it occurs with ACTH, cortisone, pregnancy, or jaundice, there is a striking increase in the histidine excretion in the urine; often 200 to 500 per cent above the control levels. So far we have been unable to reproduce this curve under any conditions other than during remission of the disease. Tolerance tests have been done, since we thought this might be nothing but a nonspecific effect on the kidney. We injected 500 mg. of histidine intramuscularly in normal individuals, who showed only a slight rise in plasma levels but no increase in urinary excretion. We do not as yet know where the increased histidine excretion comes from in remissions in rheumatoid arthritis, nor where the injected histidine goes in the normal person. Inasmuch as this is the only metabolic change we have observed constantly occurring with all types of remission studies, we believe it warrants further investigation.

R. H. Freyberg: I want briefly to discuss terminology. I believe much of our confusion surrounds the matter of terminology, particularly the word

"antirheumatic." Those who are working in the field of rheumatic disease, and particularly on rheumatoid arthritis, are concerned about the manner in which the course of this inflammatory disease can be altered favorably, so that evidences of the inflammation of the joints reduce, subcutaneous nodules reduce or disappear, the systemic evidences of disease improve, and the sedimentation rate reduces or returns to normal. If these things happen, we feel that a favorable alteration in the course of the disease has been observed rather consistently with cortisone and ACTH. Now, if that is what one means by "antirheumatic effect," that's one thing, but if one means the improvement in pain and aching by the term "antirheumatic," that's another thing. If that symptomatic improvement is referred to as "antirheumatic effect," that should be considered distinctly different from the favorable alteration in the course of this inflammatory disease.

I would like to correlate these remarks with one of the reports presented this morning. In the first slide that Dr. McGavack showed of the patients with rheumatoid arthritis, 50 per cent were indicated as improved, and in the patients with osteoarthritis, 50 per cent were indicated as improved. Now, rheumatoid arthritis and osteoarthritis are entirely different diseases; the first is a systemic disease of inflammatory nature; the second is a degenerative disease of joints, a local disease. The so-called "rheumatic symptoms" of these two diseases are very similar or indistinguishable and some of those symptoms may change, improve, or disappear simultaneously with different things that are done in the way of treatment. One cannot expect to change osteoarthritis, which is fundamentally a degenerative joint disease, but with cortisone or ACTH we have consistently seen alteration in the course of rheumatoid arthritis. Now, that is a great difference. A minor degree of improvement may occur in some of the similar symptoms which accompany each of these diseases. I do not think that we should consider every improvement in pain, for instance, as "antirheumatic," otherwise morphine is a good antirheumatic agent and we don't ordinarily consider it in this category.

I object to the reference by Dr. McGavack to osteoarthritis among the "collagen diseases." It is not so considered by the majority of investigators.

W. Bauer: If we stay here long enough we may get somewhere. I wish to make a few remarks concerning the question raised by Dr. Conn. I agree with him and with Dr. Sprague that we have available sufficient metabolic data in the case of cortisone and ACTH to permit us to conclude that alterations in electrolyte and protein metabolism can be dissociated from their antirheumatic activity. There is little to be gained from this type of study.

There are certain avenues of approach which have not been explored. Dr. Holbrook has mentioned one. Another concerns a study of the tissues involved in rheumatoid arthritis and allied diseases. These chronic inflammatory diseases are systemic in nature and affect almost

all tissues. The nature of the tissue reactions and the pathogenic mechanisms remain unknown. Knowledge concerning the physiological and chemical alterations responsible for these morphological changes would permit us to attempt reproduction of the tissue reactions. Such studies require further structural and chemical characterization of the tissues involved in rheumatoid arthritis and related diseases. This approach requires biophysical study of the fibrillar and polysaccharide components by means of electron microscopy and similar techniques, and chemical characterization of the polysaccharides and proteins of connective tissue. Once we possess information of this type concerning normal connective tissue we can examine diseased connective tissues in terms of structural and chemical alterations. For example, if we knew more concerning the nature of synovial mucin and its synthesis and breakdown, we could better interpret the altered and reduced amounts of mucin found in synovial fluid in rheumatoid arthritis. It might also be possible to understand the marked increase in synovial fluid viscosity which occurs with the administration of ACTH and cortisone. Needed information of this type cannot be gained from metabolic studies of the total organism.

I. T. Nathanson: After Dr. Dobriner's remarks I would like to suggest a technique we use that might be of some help in trying to distinguish fundamental differences in steroid metabolism of individuals with various diseases. I refer again to the patterns of excretion of the main classes of steroids after the administration of a hormone of a specific type, such as progesterone. The techniques of estimation are relatively simple. The steroid excretion pattern in patients with a variety of diseases treated in this fashion may vary considerably. It is possible that if a sufficient series of cases are studied, the steroid excretion patterns might be characteristic of a specific syndrome or at least segregate diseases that are allied on a metabolic basis. Such information, if available, may provide a springboard for investigation at a more basic level.

F. Homburger: I should like to add some data referring to the question of whether or not metabolic effects of various compounds are related to antirheumatic activity of such compounds. We have conducted metabolic balance studies in seven patients with arthritis receiving cortisone or ACTH, in whom by one means or another, we succeeded in counteracting the nitrogen loss usually caused by these hormones. In all these instances the therapeutic effect was not altered by changing the usual metabolic pattern.

J. W. Conn: I would like to add one remark to Dr. Homburger's. We have recently completed rather extensive metabolic studies with corticosterone, Compound B, in normal individuals. We observe definite metabolic changes both in electrolyte metabolism and in organic metabolism, namely, the production of negative nitrogen balance, increased uric acid excretion, increased uric acid-creatinine ratio, etc. Dr. Robinson

of our arthritis clinic has used that material in a single individual, in a dose of 200 mg. a day for 10 days, with essentially no antirheumatic activity at all. So, here is a compound of the 11-oxygenated variety that does have metabolic activities of both desoxycorticosterone and cortisone combined in a single steroid and still proves to have very little or no effect upon rheumatoid arthritis at that level of dosage.

R. G. Sprague: I would like to extend Dr. Conn's remarks concerning Compound F and Compound B, to Compound A acetate. We have recently extended the previous studies of this compound to another patient with rheumatoid arthritis, employing a dose of 500 mg. daily for 18 days. At this dose level there was a rather marked electrolyte effect, but no evidence of antirheumatic activity.

There has been a good deal of comment here about the necessity for studies of absorption of steroids of low solubility. Dr. Dobriner has expressed appreciation of the very nice studies of Dr. Ascoitia. I would also like to express appreciation of these important studies and point out that there is also a need for studies by our biochemical colleagues of the absorption of these compounds when administered orally. There is already good evidence, first obtained by Dr. Freyberg, that cortisone administered orally is absorbed promptly and, judging by clinical criteria, quite completely. We have found that cortisone administered orally has much more prompt metabolic effect than when it is administered intramuscularly. Obviously, we cannot properly appraise the metabolic or therapeutic activity of any compound, whether administered orally or intramuscularly, without information concerning its absorption.

T. H. McGavack: In the slides shown by Dr. Robles Gil, periods of pregnenolone therapy always followed immediately upon periods of therapy with ACTH. Since it is known that the pituitary may be depressed at such times and the responsiveness of the adrenal lessened, I am wondering if one can by this procedure adequately appraise the action of any new drug, such as pregnenolone, under these circumstances.

The question of sedimentation rate as an index has been referred to repeatedly in today's discussions. Perhaps our experience is inadequate but we have seen cortisone and ACTH produce maintained improvement without changes in the sedimentation rates, but this has not happened as frequently as with pregnenolone. The comments of Dr. Sprague, Dr. Dobriner, Dr. Nathanson, and others all bespeak the necessity for common denominators for measuring the efficacy of steroid hormones in rheumatoid arthritis, if we can find them. Perhaps in my presentation I did not emphasize one point, namely, that the condition of the individual may have something to do with the response obtained. It seems to me in some of the careful studies that have been presented here on external balances, no satisfactory conditioning factors whatsoever have been introduced, and I am wondering if they may not be necessary to bring out certain actions that may prove useful under specific conditions.

In response to Dr. Freyberg's remarks, I do not recall stating or implying that osteoarthritis was a collagen disease; if I did, I am glad he has afforded me an opportunity to correct such an impression. I do emphasize the point that Dr. Smith stressed. As far as we are concerned, pregnenolone produces most of its changes in the periarticular, systemic, and psychogenic manifestations. Dr. Dobriner and Dr. Bauer brought up the point that rheumatoid arthritis, lupus erythematosus, and kindred conditions should not be considered as collagen diseases. I think we might also bring up the further point that the composition of collagen normally changes from youth to old age and contains at one period certain amino acids not present at another. This introduces still another variable into this problem of evaluation.

I would like to ask Dr. Holbrook whether his balance tests for amino acids were made in normal subjects or in those with rheumatoid arthritis?

W. P. Holbrook: Further tolerance tests are being studied by different methods in normal persons and in patients with rheumatoid arthritis.

Concluding Comments

J. W. Conn: Shortly after I arrived in Mexico City, I was presented with the proposition that Dr. Rhoads would be unable to be present at the meetings and that the Committee on Arrangements had selected me to substitute for him in summarizing, evaluating, and recommending future investigation based upon the clinical papers presented at this conference. It was pointed out that I am not identified with the field of rheumatology; that I was regarded as being able to evaluate research efforts of other people, and that I would, therefore, be an impartial observer. Because I realized that this would be an unenviable position for anyone to find himself in, I suggested several other members of the conference. I was overruled and accepted the assignment reluctantly.

This combined meeting-vacation, as we are all aware, has been one of the most delightful and hospitable ones that I have ever had the privilege of attending. For this, we owe a debt of gratitude to both our North American and Mexican friends who are our hosts. But science is science in any country and in my personal evaluation of the results of this meeting, I intend to call a spade a spade and let the chips fall where they may. I intend not to point a finger at any one investigator but it will be clear that I do not accept as fact the conclusions of some. I trust that my medical colleagues will listen to my evaluation, whether it is right or wrong, in the spirit in which it is given. I harbor no personal animosity against anyone. This is a cold-blooded, objective, interpretation for which I alone am responsible. My comments, therefore, will be brief and pointed.

The purpose of this meeting is to evaluate the status of the 11-desoxy steroids, and my assignment is to evaluate the clinical status at present. I see no need to review individual papers and to quote again conflicting statistical information based upon clinical observations of individual

observers. I shall go directly, therefore, to my personal overall evaluation of the present status of the 11-desoxy steroids in clinical medicine. This excludes any evaluation of the naturally occurring sex hormones and of desoxycorticosterone. First, I have seen nothing in the papers presented at this conference which convinces me that pregnenolone, or the other 11-desoxy steroids tested, have any metabolic activities when administered to man either orally or parenterally in the doses that have been given. Perhaps the correct things have not been measured but of the things that have been measured, none has shown significant change in my opinion. But I mentioned in the course of the conference that lack of metabolic activities as we measure them today need not eliminate the possibility of a clinical effect based upon a change which we cannot as yet measure chemically as, for example, the antirheumatic effect. This brings me to my second point.

I have seen nothing in the papers presented at this conference which convinces me that pregnenolone, or the other 11-desoxy steroids tested, have any effect upon rheumatoid arthritis or upon any other clinical entity in which they have been tried in the present dosage schedule. I interpret the enthusiastic reports of some of the essayists regarding the effectiveness of pregnenolone in rheumatoid arthritis as being due to factors other than the effects of the pregnenolone which was administered; elements such as the natural course of the disease, psychotherapy, and other factors. Those workers who report negative results with pregnenolone in rheumatoid arthritis have observed that placebo administration produces slight symptomatic improvement in about the same degree as that produced by pregnenolone. I am not convinced that pregnenolone, or the other 11-desoxy steroids, have actually ever been tested in man. When given orally, we know nothing of how much is absorbed and how much goes out in the feces. When given parenterally in man we know nothing about the rate of absorption or the fate of the injected material. It may well be that pregnenolone activity in man is still untested and that as such it still has the status of hundreds of other untested compounds. That to date no untoward effects have been observed with pregnenolone may also be related to this same factor. Pregnenolone may well be a naturally occurring intermediate in testicular tissue, as Dr. Selye reported, and it may be a natural intermediate in the *in vivo* synthesis of adrenal steroids, as suggested by the report of Dr. Zaffaroni. This has no bearing on whether or not exogenously administered pregnenolone is effective against disease in man.

Recommendations for future investigation:

1. It is my opinion that, for the present, a major share of the research facilities at our disposal should be directed toward a study of the mechanisms of action against disease, of compounds of proved value, like cortisone, rather than to spend too much time attempting to screen compounds which are at best of only questionable value. For when we have solved the mystery of cortisone and ACTH, we shall have gone a long way in our screening program with the probable elimination of hundreds

of compounds from such a testing program. The question of the methods by which we are to approach the problem of the mechanism of action of ACTH and cortisone is beyond the scope of this meeting. This will undoubtedly eventually be settled by the enzyme chemist. In the meanwhile such approaches as Dr. Bauer's observation of changes in polymerization of polysaccharides in joint fluid under the influence of cortisone and ACTH, an observation confirmed by Dr. Robinson of our group; or the approach of Dr. Holbrook on the changes in amino acid excretion; or the approach of Dr. Dobriner on abnormal excretion patterns of steroids; all these are hopeful.

2. A portion of the research facilities of those interested in arthritis could well be engaged in a screening program of the many 11-desoxy compounds available.

3. An important line of investigation of the potentialities of 11-desoxy steroids I envision as follows: There appears to be increasing evidence that competition for a common substrate exists between some steroid compounds. It may well be that synthetic and unnatural steroidal compounds can be screened against a variety of common diseases which are in themselves thought to involve excessive activity of natural steroids, or to involve the production of abnormal steroids. It is my belief that this type of investigation may yield important benefits to man. Finally, in predicting future uses of desoxy steroids, Dr. Selye stated that these compounds should not be expected to do what ACTH and cortisone do, but should be expected to do opposite things, for example, the *promotion* of connective tissue proliferation. He suggested potentialities for these compounds in those diseases which have as their requirement connective tissue proliferation and encapsulation.

Two days ago, because I felt a growing sense of responsibility in the position of evaluator, which I had accepted against my better judgment, I asked two other members of this conference to be prepared with their evaluation of this meeting. This was an effort on my part to divide the responsibility and to give you the advantage of independent appraisals. These men I selected because they too are not rheumatologists and because the conference at large respects their opinions. I wish to emphasize that I have no idea of what either of these men will say. The three of us have not conferred at all. The men are Dr. Randall Sprague and Dr. Ted Danowski and they are prepared to evaluate for you.

R. G. Sprague: As Dr. Conn indicated, his opinions and those which I will express were arrived at independently. In spite of this, there is a broad area of overlapping of what we have to say. I can argue with very little that Dr. Conn has said. A few further comments on certain points are in order.

The first point is the appraisal of the therapeutic utility of the 11-desoxy steroids which have been discussed at this conference, with special reference to their effects on rheumatoid arthritis. I can only conclude

from the evidence I have heard presented here that these steroids have little, if any, antirheumatic activity. In trying to reconcile the discrepant results which have been reported, one must consider two major sources of variations, the observer, on the one hand, and what is being observed, on the other. In my opinion, the observer is the most likely source of discrepancy. This does not imply that observations were not made honestly and thoroughly in all instances, but it does suggest that some observers assigned more significance than others to certain types of slight improvement, usually subjective, in their patients.

In my opinion, in addition to a lack of significant therapeutic effect, no convincing evidence of any type of physiologic activity of the 11-desoxy steroids under discussion (except the sex steroids), in human subjects, has been presented in these sessions. (Addendum: This applies to the studies of the effects of pregnenolone on spermatogenesis in human subjects presented in the last session.) This, of course, does not imply that these compounds are totally devoid of physiologic activity, particularly since there is not sufficient information about their absorption and since there is evidence of some types of physiologic activity in animals.

Certainly existing knowledge of the absorption of the compounds, whether administered orally or intramuscularly, is meager. The studies of Dr. Ascoitia suggest that poor absorption of pregnenolone and 21-acetoxypregnenolone from the sites of injection is a possible factor in their virtual lack of therapeutic and metabolic activity in human subjects. In any event, whether or not the apparent inert character of these compounds is related to poor absorption, it is my personal belief that attention should now be directed to other compounds, and when I say other compounds, I would include absorbable esters of pregnenolone and also esters of sufficient solubility to permit intravenous administration.

A coördinated steroid testing program seems desirable since cortisone and ACTH, because of the broad scope of their activity, still leave something to be desired in the treatment of rheumatoid arthritis and allied diseases, particularly in the hands of the inexperienced. There seems to be little reason to expect that Compound F will differ greatly from cortisone from a therapeutic or physiologic standpoint. In a program of testing, I believe that compounds should be sought which have an order of therapeutic activity which manifests itself promptly and is readily recognizable. If this premise is correct, a screening program should not require the use of large numbers of patients nor prolonged periods of administration, provided, of course, that the compounds are known to be absorbable. More careful appraisal of more limited numbers of patients than have been studied thus far will yield more profitable results than less careful observation of larger groups of patients. Furthermore, the use of large doses is desirable in the early investigation of compounds of known activity. Adherence to this principle will save labor and material in the long run. It was this principle that led Drs. Hench, Kendall, Slocumb, and Polley, in their initial studies of the effects of cortisone in rheumatoid arthritis, to employ a dose of 100 mg. daily, which at that

time was regarded as a large dose. If they had administered smaller doses the effects might well have been missed.

In evaluating the antirheumatic activity of new compounds, let us not forget the sedimentation rate. This is an objective test. I will personally question the therapeutic efficacy of any agent which does not cause a marked reduction in sedimentation rate in a high proportion of cases.

What is the possibility of dissociation of antirheumatic and other physiologic activity? At present this is only a hope. Evidence presently available points to an association rather than to a lack of association of antirheumatic and other types of physiologic activity, some of them undesirable from a therapeutic standpoint. However, Dr. Lipschutz' observation this morning that certain steroids may exhibit antifibromatogenic activity independent of any other known activity gives reason to hope that antirheumatic activity may also exist almost totally divorced from other types of activity. It is to be hoped that a compound with a maximal amount of antirheumatic activity and minimal other activity will some day be found. Such a compound has not been described at this conference. The finding of a compound meeting these specifications should, of course, be a major objective of any proposed testing program.

T. S. Danowski: I still recall with pleasure the kindly elderly lady who patiently listened to me ask her about her diabetes when I was a medical student. She answered my questions courteously when I inquired as to whether she was testing her urine, following her diet, etc. When I finished, she looked at me and said, "Young man, I had diabetes before you were born." And a very rapid mental calculation indicated that she was quite right, and she was objecting to the fact that I was perhaps expressing too much interest in her diabetes. At the same time, I recall how vigorously we fought the admission of arthritic patients to the medical ward a decade ago. They had to be brought in under various kinds of somewhat dishonest stories before the resident would allow them to come into the medical ward. All that is changed. The rheumatoid arthritics of our country, and elsewhere, now have the feeling that they are part of the Metropolitan Opera. As a new compound is tested, they are surrounded with solicitude and every bit of urine is carefully saved and studied. You will recall that we heard, during these past few days, that certain of them were brought in and put into a wheelchair under the physician's personal supervision, and I venture to state that this very personal interest in the welfare of these hitherto neglected patients must have done something to them. I cannot believe, after meeting with my colleagues at this conference, that those who reported no improvement whatsoever were so totally devoid of charm that they failed to elicit a favorable reaction from at least some of their patients. Perhaps it would be unfair to state that they enchanted 50 per cent of them, so that we are left with the problem of deciding whether there may be still some

property of these compounds that produces a beneficial effect. One of the real problems that has faced us is that we have tried to match these compounds against wonder drugs and, as Dr. Sollins remarked yesterday afternoon, perhaps they should be matched against aspirin. It is true, as Dr. Conn and Dr. Sprague have pointed out, that no one has presented any evidence up to this time that these compounds exert any physiological effects in these patients. On the other hand, we cannot ignore the evidence that pellets of these steroids do decrease in size when placed subcutaneously, that not everybody has hard nodules, and perhaps when you incise them you do not recover all of the pregnenolone. Perhaps some of it is being absorbed and perhaps some of it is exerting a physiological effect.

Now I shall do a very unfair thing because perhaps this isn't the time to introduce new checkers into the game but I did get an air-mail letter today which represented a statistical analysis of stool data on patients receiving 21-acetoxypregnenolone. The sodium and the potassium, the chloride and the nitrogen in stools of these patients, and a large series of controlled patients, some 30 in number, were calculated as per day output, per 100 gm. stool output, and per 1 gm. of nitrogen output. We have studied the stools not only of control subjects but of subjects receiving cortisone, ACTH, or the two compounds together. There is no evidence, statistically or by direct inspection, that the administration of cortisone or ACTH alters the stool output of sodium, of potassium, of chloride, or of nitrogen per day, per 100 gm. of stool or, in the case of the first three, per 1 gm. of nitrogen. On the other hand, the patients receiving 21-acetoxypregnenolone, and I must admit there are only seven observations, showed a statistically significant decrease in the daily output of nitrogen and in the nitrogen put out per 100 gm. of stool. They showed a statistically significant decrease in stool sodium per day and stool sodium per 100 gm. of stool. There was no change in the sodium output per gram of nitrogen but you would not expect this because inasmuch as the stool nitrogen itself fell, a proportionate drop in sodium and nitrogen would not alter this ratio. There was no change in the amount of potassium put out per day or per 100 gm. of stool but there was a relative increase in the amount of potassium put out per gram of nitrogen; again the potassium didn't change, but since the nitrogen fell, the ratio rose. The same is true of chloride. Now the thing that especially excited me was that we have an even smaller number of patients who received desoxycortisone acetate, only four in number, but these patients showed the same kind of change that the 21-acetoxypregnenolone patients showed. This may be of course a series which is selected, a series which upon enlargement will fail to show these differences, but this may also be a little bit more indirect evidence that perhaps some of the compounds that have been given in the pregnenolone series have been absorbed and have modified the functions of the membrane lining of the gastrointestinal tract. This does not alter, of course, the facts that have been pre-

sented to us during the last several days and, certainly, I would agree with the two gentlemen who preceded me that we have not convinced ourselves that pregnenolone exerts any distinctive beneficial effect in rheumatoid arthritis. I wonder if we would feel the same way if we matched it not against cortisone and ACTH but against aspirin.

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The Adjuvant Use of Various Steroids in Relative Seminal Inadequacy in the Human with Particular Reference to Pregnenolone

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Infertility may be defined as the inability of a couple to conceive after a year of normal marital endeavor. If investigation proves that the female is ovulating at fairly regular intervals, that the cervical mucus is favorable for sperm penetration and maintenance at about the time of ovulation, that the tubes are patent and physiologically motile, that her metabolic function, constitutional status, and psychosomatic balance are within normal limits, then one may assume that the fertility potential of the female is probably adequate.

On the other hand, seminal inadequacy indicates that one or more factors in the semen do not measure up to what is considered as "normal"; i.e. adequate for impregnating a potentially fertile woman. Although several men have attempted to set up minimal "normal" standards (Meaker,[1] Williams,[2] Charney,[3] Hotchkiss,[4] and Weisman[5]), we shall utilize the following criteria established by the Research Correlating Committee of the American Society for the Study of Sterility.[6]

Following a sexual rest of from three to four days, a semen specimen is obtained by masturbation or coitus interruptus directly into a clean glass jar. At least two, and preferably three, individual specimens should be evaluated. In brief, the minimal "normal" values specify that the seminal ejaculate should liquefy in from 10 to 30 minutes, with an average volume of 2 to 4 cc. and with a minimum count of 40 million spermatozoa per cubic centimeter, or a minimal total of from 125-150 million. Each count should be done in duplicate. There should not be more than 20 per cent abnormal heads on the stained smear. Motility—or purposeful progressive motion—should be present in at least 25 per cent of the sperm eight hours post-ejaculation at room temperature.

On the basis of the foregoing, seminal inadequacy in this clinical study was judged to be present if on repeated examination, one or more

of these factors remained constant: (1) a count of less than 40 million per cubic centimeter, (2) abnormal sperm-head forms of over 20 per cent, and (3) motility of less than 25 per cent at eight hours post-ejaculation at room temperature. Recently, motility percentage has been correlated with the live-dead sperm stain of Blom.[7]

In addition, from a clinical point of view, since none of these men had been able to impregnate a potentially fertile wife, further weight was added to the diagnosis of relative seminal inadequacy. The term "relative" is used advisedly, since (1) there are as yet no definite standards by means of which a semen specimen can be judged inadequate except in the complete absence of spermatozoa, and (2) it is more than feasible for a man with even extreme oligozoospermia to impregnate a woman who is unusually fertile.

The present study was undertaken some seven years ago after it had been found that various gonadotrophins, such as chorionic gonadotrophin with or without "synergists," equine gonadotrophin, and various extracts of the adenohypophysis not only failed to improve the seminal picture, but frequently reduced the sperm count very significantly. In a few cases, the damage seemed permanent.

At this time the thought that various steroids might have a direct trophic influence upon spermatogenesis was stimulated by the report of Leathem and Brent[8] as well as by the previous papers of Selye et al.[9] and Nelson.[10-12] Finally, the review of Masson[13] on the spermatogenic activity of various steroids in the rat prompted our group to attempt to improve the fertility potential of men with seminal inadequacy by the adjuvant use of various steroids as they became available to us. Among those screened were testosterone, methyl testosterone, ethinyl testosterone, progesterone, and more recently pregnenolone and methylandrostenediol.

Clinical Studies

The men studied in this group were the husbands of wives who had consulted us originally for infertility. The husbands were followed carefully by their family doctor or urologist. All semen studies, with rare exception, were performed in one laboratory where all procedures are those recommended by the American Society for the Study of Sterility.[6]

Complete work-up was secured in each case and, with a rare exception, at least three semen specimens from each were evaluated. One of these was obtained at least a month after the previous one. In some cases as many as 12 pretreatment studies were made. Whenever a steroid was added to the therapeutic regimen, no other change was made.

Testosterone Propionate. Testosterone propionate dissolved in sesame oil was injected in eight cases at a weekly dose range of 5 mg., 10 mg., and 25 mg. for periods of four to twelve weeks. Evaluation of the semen was repeated at monthly intervals wherever feasible. The results are presented in Table I. In brief, it was observed that the dose range of 5 to 25 mg. a week had practically no effect upon the sperm concentration in these subjects. With the higher dose range, however, there was a slight

but definite tendency toward the diminution of large immature forms and thus a decrease in percentage of abnormal forms. In one case there was some improvement in the duration of motility. This was the only case where pregnancy occurred.

The results obtained with testosterone propionate indicated that perhaps a related steroid might prove to be a more potent spermatogenic stimulus either in the direction of improving motility, increasing the duration of purposeful motility, or decreasing the number of abnormal forms.

Methyl Testosterone. This steroid was administered orally in doses of 5 to 10 mg. daily for a period of four to eight weeks followed by a rest period of four weeks. More recently the buccal or sublingual form has been utilized. Ten cases were studied. In general, the results obtained

TABLE I

EFFECTS OF TESTOSTERONE PROPIONATE ON SEMINAL INADEQUACY

Dose per Week, Mg.	*Number of Cases*	*Number with 50% Increase in Count*	*Number with 100% Increase in Motility*	*Decrease in Abnormal Forms**
5	3	0	0	0
10	3	1	0	1
25	2	0	1	1

* Large immature forms.

TABLE II

EFFECTS OF METHYL TESTOSTERONE ON SEMINAL INADEQUACY

Dose per Week, Mg.	*Number of Cases*	*Number with 50% Increase in Count*	*Number with 100% Increase in Motility*	*Decrease in Abnormal Forms**
35	5	1	1	2
70	5	1	2	1

* Large immature forms.

(Table II) disclosed a decrease in abnormal forms, particularly the large immature forms. There was suggestive, but not conclusive improvement in three cases (30 per cent), insofar as increase in motility was concerned; an increase in sperm population was observed in two cases (20 per cent). Two pregnancies occurred, but one woman miscarried at twelve weeks and the other at seven weeks.

At present, methyl testosterone is utilized primarily where there is a preponderance of large immature forms and then practically always in conjunction with thyroxin, as there appears to be a synergism in the activity of these hormones.[14]

Ethinyl Testosterone (anhydrohydroxyprogesterone). This compound was administered orally in daily doses of 5, 10, 20, and 30 mg. for a period of four weeks, and occasionally for eight weeks, in five cases of seminal inadequacy. No positive response was apparent as far as count, motility, or morphology were concerned. Recently, daily doses of 10 to 20 mg. sublingually for a month yielded similarly negative results.

Progesterone. Progesterone dissolved in sesame oil was administered in dosages of 5 and 10 mg. per week for four to twelve weeks to four men. No specific response could be elicited insofar as the seminal inadequacy was concerned.

When progesterone was given in daily doses of 5 mg. sublingually or in buccal tablets daily for periods of four to eight weeks (three cases), again no changes could be detected.

Desoxycorticosterone Acetate. The steroid was administered in oil at dosages of 5 to 10 mg. per week for a period of four weeks in two cases and eight weeks in one case. No changes were detected.

Pregnenolone* (Δ^5-pregnene-3β-ol-20-one). Since September 1947 when this steroid became available to us, a large number of men have been treated with this substance. In each case, the failure to conceive was felt to depend primarily upon the seminal inadequacy. In the entire series, infertility had existed for an average of over three years. In practically every case at least three individual semen specimens had been evaluated, and in many instances six to twelve or more seminal ejaculates were studied. Many patients had been treated previously elsewhere with gonadotrophins of varying sources, thyroid extract, diet, crude liver concentrates, vitamin E, and the vitamin-B complex, as well as miscellaneous "shotgun" preparations.

Whenever possible, semen specimens were studied at four- to six-week intervals. Pregnenolone was administered by three routes: (1) orally in dosages of 50 to 100 mg. daily, (2) intramuscularly in aqueous suspension (50 to 100 mg. per cubic centimeter), and (3) intramuscularly as pregnenolone acetate in sesame oil (20 to 50 mg. per cubic centimeter).

1. Oral pregnenolone—Since the efficiency of absorption of this substance from the intestine was not definitely known, it was used in only five cases.

2. Pregnenolone in aqueous suspension was given in 14 cases. In 10, there occurred so much tenderness and local tissue reaction, including two sterile abscesses from which a large quantity of crystals (pregnenolone?) was obtained, that this form of administration was discontinued. In only four cases was a complete series of injections given.

3. Pregnenolone acetate dissolved in sesame oil became the vehicle of choice. After much trial and error the following dose schedule is being utilized at present: 50 mg. weekly for four weeks, then 50 mg. twice a week or 100 mg. weekly for four to eight weeks, followed by a four-week rest.

* These data on pregnenolone were first reported at the Sixth Annual Conference of the American Society for the Study of Sterility, San Francisco, June 25, 1950.

A second series of injections consists of 100 mg. per week for four weeks, followed by 200 mg. a week for four to eight weeks.* To date, 40 men have been followed sufficiently to permit adequate evaluation.

Results with Pregnenolone Therapy

1. Oral—Of three patients who were followed adequately, one showed a marked increase in sperm population from a pretreatment level of 25-37 million per cubic centimeter to 60-74 million per cubic centimeter, associated with a marked improvement in duration of motility. Surprisingly, these improvements were maintained for as long as six months

TABLE III

EFFECTS OF PREGNENOLONE ACETATE ON SEMINAL INADEQUACY

Highest Sperm Count in Millions per Cc. before Pregnenolone Therapy	*No. of Cases*	*No. of Cases Showing a 50% or Greater Improvement in Count Following Therapy*	*No. of Pregnancies**
1– 9	4	2	2
10–19	5	4	2
20–29	9	4	6
30–39	7	3	4
40–49†	4	3	2
50 plus†	11	3	6
Total	40	19	22

* Four men succeeded in impregnating their wives twice.

† Included in these groups were cases of oligozoospermic men whose usual count ranged from 5-30 million per cubic centimeter but who would on rare occasions, perhaps once in 3 to 10 specimens, produce an ejaculate of 60-140 million per cubic centimeter.

after therapy was stopped. In a second patient, the duration of motility increased while the sperm count showed a decrease of from 45-55 million to 35-40 million per cubic centimeter at eight weeks, but a month after cessation of therapy, the count returned to pretreatment levels. In the third patient, no changes were noted.

2. In the four patients receiving the aqueous suspension for at least one full series of injections, no significant changes were noted. This conclusion is not completely valid, however, since these men became rather unco-operative in completing the series, thus making the period of observation inadequate.

3. In the 40 patients treated with pregnenolone acetate in oil, the results proved to be promising. They may be summarized as follows:

Count. With respect to sperm population, 19 men (40 per cent) showed an increase of 50 per cent or more (Table III). In five cases (16 per cent) the count showed a decided decrease. In four instances, this

* If the semen does not improve in 8 to 12 weeks, then the next higher dose level is used in the next series of injections.

was noted when the weekly dose exceeded 200 mg. a week and in one case it occurred at 100 mg. per week.

In the 25 cases of persistent oligozoospermia (1-39 million per cubic centimeter) the sperm count definitely increased in 13 cases or 52 per cent (Table III).

Motility. This is a difficult end point to assess under the best of conditions, since it must depend upon the judgment, skill, and experience of the observer. Not infrequently two skilled observers may vary by 10 per cent or more. In any event, we have included only those observations in which the change was clearcut and definite and in which at least a 100 per cent improvement had occurred. For various reasons of our own, we have chosen the percentage motility at the end of eight hours at room temperature as the most critical end point. By this standard there was a very distinct improvement in 14 cases, or 35 per cent.

TABLE IV

NUMBER OF MONTHS FROM START OF PREGNENOLONE ACETATE THERAPY TO SUCCESSFUL CONCEPTION

Months	*Pregnancies*
1	3
2	4
3	4
4–6	6
7–9	3
10–12	1
12 plus	1
Total	22

Morphology, Viscosity. No definitive changes in morphology could be demonstrated. Similarly, inconclusive results were obtained in observations of the volume of the ejaculate and its viscosity. In two instances there was noted a definite diminution of crystals in the semen.

Conceptions.* Eighteen of these treated husbands were successful in impregnating their wives, four being successful twice (Table III). Thus, 22 pregnancies occurred. Of these, eight women aborted and 14 were successfully delivered of a living child. No evident congenital abnormality was found. The salvage rate of 64 per cent is about the average success in our practice for infertility in couples. Of every 100 infertile couples who eventually conceive, we have found that our overall salvage of a living baby is approximately 70 per cent compared with the normally fertile couples group, in which about 86 per cent will have a living child.

Table IV shows the number of months elapsing between initiation of

* The diagnosis of pregnancy was made by means of at least two, and usually three, criteria: basal body temperature curves, positive male frog test, and pregnandiol excretion. However, a positive pelvic examination remained the ultimate criterion in diagnosing pregnancy.

pregnenolone acetate therapy and successful conception. Of these 22 pregnancies, 17 (77 per cent) pregnancies resulted within six months of therapy, with 11 (50 per cent) occurring in three months. In 18 cases, definitive improvement in the semen picture (motility, duration and/or count) occurred in only nine cases. On the other hand, since nine others were also successful, the fertilizing capacity of the semen may have been enhanced in another as yet undetermined manner.

Of the many interesting cases observed, the following is unusually significant. The wife had been found to be potentially fertile, while the husband was found to have a lowered basal metabolic rate (minus 17) and a vitamin-B complex deficiency; repeated semen analysis revealed a count of from 2–8 million per cubic centimeter with no viability after four hours. Actually, this couple had sought donor artificial insemination since two doctors had advised the husband, after a series of gonadotrophin treatments and liver injections, that he would never become a father. After eight months of thyroid extract and thyroxin, and a proper diet fortified with added vitamin-B complex factors, the basal metabolic rate became normal (plus 1), while the semen disclosed a sperm population of from 15–20 million per cubic centimeter. Motility had improved to 10 per cent at eight hours. The fourth month after the start of a series of pregnenolone acetate injections, his wife became pregnant. The sperm count had risen to 25–34 million per cubic centimeter, while the motility improved to 20 per cent at eight hours. She aborted a blighted ovum at 10 weeks.

Since the semen remained the same, no further pregnenolone acetate was given. After six months, when pregnancy did not intervene, a second series of pregnenolone acetate injections was started. The semen picture changed only in that the husband's count dropped somewhat to from 12–18 million per cubic centimeter. The wife became pregnant three months later and at present is 35 weeks pregnant.

This case certainly points to the fact that pregnenolone acetate apparently enhances the fertilizing capacity of the spermatozoa. The modus operandi, however, remains to be elucidated.

Constitutional Effects of Pregnenolone Acetate:

Libido. Several of the wives volunteered the fact that after one to three months of pregnenolone therapy there was a definite increase in the husband's libido. In five cases the increase was most decided.

State of Wellbeing. About half of the men noted a definite increase in their sense of wellbeing.* They felt better and seemed to fatigue less easily. This euphoria was most difficult to assess, since the male could

* This euphoria stands out in great contrast to the effects of pregnenolone in women. In three cases of severe premenstrual tension, where progesterone had been successful in alleviating symptoms, pregnenolone acetate was substituted—50 mg. per week for two weeks beginning the fifteenth day of the cycle. In each case the tension was not only not relieved, but in two cases the irritability was remarkably aggravated. One of these women noted an unusually great premenstrual weight gain (6.5 pounds). In addition, two women with essential dysmenorrhea claimed that pregnenolone had caused them to have some of their most painful periods.

easily assuage his guilt feelings by the fact that something positive was being done for him. This could still be true even though we were most careful to point out that this therapy was being given in an attempt to increase the duration of viability, that it would not increase his count, and that it only worked in a small percentage of cases.

Along with the sense of wellbeing it was noted that several men gained from 5 to 10 pounds of weight during therapy. It is recognized that an increased appetite could easily accompany an increased sense of wellbeing. On the other hand, pregnenolone may stimulate increased nitrogen retention.

Correlation of Lowered Basal Metabolic Rate and Pregnenolone Therapy. Early in our studies a most interesting phenomenon was observed. In one case of oligozoospermia with a lowered basal metabolic rate (B.M.R.) of minus 17 per cent, pregnenolone had no effect upon the seminal inadequacy. The individual was placed upon thyroid extract, 1 grain daily. Two months later the basal metabolic rate measurement showed no significant change. The dose was increased to 2 grains daily with no change in the basal metabolic rate or seminal picture. Pregnenolone acetate was given in two series with no change in the constituents of the semen.

The subject was then placed on thyroxin, 0.5 mg. daily, and three months later his basal metabolic rate was plus 1. Semen remained essentially the same. A third series of pregnenolone acetate was started at this time with no increase in dosage. Up to this time the sperm count had ranged from 19–31 million per cubic centimeter, with 5 to 10 per cent motility at eight hours (seven specimens). A month after pregnenolone acetate was started, the count had increased to 43 million per cubic centimeter, with a motility of 20 per cent. Two months later the count was 103 million per cubic centimeter, with 50 per cent motility at eight hours. His wife skipped her next period and now they are the proud parents of a baby boy.

We have seen this phenomenon repeated many times. At present, pregnenolone is not given until the basal metabolic rate is about minus 5 or higher. The evidence at hand seems to point to a synergism between pregnenolone and thyroxin upon spermatogenic function. Thyroxin may be likened to "ethyl" in ordinary gasoline in that it probably allows for a more efficient metabolic utilization of the steroid.

General Conclusions

Until recently the concept that the internal secretion of an endocrine gland could not stimulate that gland was held to be a universal truth; but, slowly at first, and now to an increasing degree, the evidence is accumulating that the endocrine secretion of the gonad in particular, is definitely an integral metabolic unit in its overall functional activity. Burrows[15] has recently reviewed these data.

From the evidence at hand, certain androgenoid* steroids may not

* Androgenoid—literally, to make like a male.

only maintain, but actually stimulate, spermatogenesis in hypophysectomized male animals. This was first demonstrated in 1934 by Walsh, Cuyler, and McCullagh,[16] who showed that androsterone could maintain spermatogenesis. This was confirmed by Nelson and Gallagher,[10] while Nelson and Merckel[11] later demonstrated similar spermatogenic activity for androstanedione, androstanediol, testosterone, dehydroandrosterone, and *cis*-androstenediol. The first two compounds were the most potent of the substances studied. Additional confirmation has been presented in the rat[17, 18] as well as in the rabbit[19] and guinea pig.[20] In the hypophysectomized monkey, Smith[21] found that testosterone and androstenedione would not only maintain spermatogenesis but, if administered some time after operation to permit testicular atrophy, would actually re-institute active spermatogenesis (see also Green and Zuckerman[22]). Furthermore, Nelson and Gallagher[10] were able to mate successfully their steroid-treated, hypophysectomized male rats.

Cutuly et al.[17] believe that these spermatogenic steroids exert their influence by maintaining the testes in the scrotum, but Gaarenstrom and Freud[23] believe that testosterone, at least in the rat, is necessary for mitogenesis in the tubular epithelium. In a later publication, Gaarenstrom and de Jongh[24] state that testosterone is essential for maintaining the cell columns of the seminal tubules in such a way that spermatogonia may evolve into spermatozoa. When testosterone was placed as a pellet directly into the testes, spermatogenesis was stimulated in a local area only.[18, 21]

Perhaps the spermatogenic stimulus of these steroids may be explained by placing the seminiferous tubules in the same class as the accessory male organs such as the prostate and seminal vesicle.[15] Like these organs, the tubules are stimulated by gonadotrophins only secondarily to the effect of the latter upon the interstitial cell to produce androgenoid substances.

In the human, reports of a stimulating influence of testosterone upon the testes as well as on spermatogenesis have appeared from time to time.[25, 27] Our failure to demonstrate more positive results in our early work may have been due to inadequate dosage or to the short term of the studies. On the other hand, Heckel[28] has reported an overwhelming suppression of spermatogenesis in man by this steroid. When his data are carefully analyzed, however, it is noted that suppression occurs only after continued therapy over a period of three or more months with relatively massive doses of the hormone. It is felt that his conclusions are not entirely justified by his data. In fact, in two of his cases, spermatogenesis was apparently stimulated.

Recently, Heller et al.[29] have reported that the human testes show an initial severe depression histologically, as a result of large doses of testosterone. This damage lasts from six to twelve months but is almost always followed by such a strong rebound phenomenon that in practically every case in which the testes showed previously poor function, subsequent biopsies from six to eighteen months later disclosed excellent anatomic evidence of good spermatogenesis.

Pregnenolone was first isolated from hog testes in 1943 by Ruzicka and Prelog.[30] Selye[9] has reported extensively on its various effects, stating that it possessed marked spermatogenic activity as well as a protective action on the testes against damage by estradiol. Some feel that since estrogen is present in the testes in large amounts, the simultaneous presence of pregnenolone may be more than coincidental.[30] In the rat, pregnenolone possesses spermatogenic activity;[13] however, no striking results were obtained in the human insofar as oligozoospermia was concerned, although some increase in motility duration seemed to occur.[31] Henderson et al.[32] have recently presented a complete review of the various aspects of pregnenolone.

The exact mechanism by which pregnenolone may exert a helpful influence upon seminal inadequacy in the human remains to be determined. It cannot be denied that its action may be based on a constitutional effect. The suggestion has been offered that the fertilizing capacity of the sperm is perhaps enhanced by some changes in enzyme systems, or that fructose secretion is stimulated.[33]

The synergistic action of thyroxin and pregnenolone is one which merits further study. The synergistic effect of thyroxin with other hormones, however, does not leave this an isolated phenomenon.[14] For example, we have encountered a number of climacteric women whose symptoms were not relieved by estrogenoid substances until after thyroid extract was administered. It was the remarkably keen clinician, Dr. Peter Marshall Murray, who first pointed this out to us many years ago. Thyroxin may act as a nonspecific metabolic accelerator, or this synergistic effect may be mediated through other channels.

The number of pregnancies that followed pregnenolone acetate therapy forms a rather solid positive answer to its apparent beneficial effects upon relative seminal inadequacy. On a clinical basis, the 25 cases of oligozoospermia might have yielded some two to four pregnancies over the course of several years, even without therapy. With the adjuvant aid of pregnenolone, 14 pregnancies ensued. More than just chance alone is obviously involved. Certainly the fertilizing capacity of the spermatozoa must have been markedly improved, since in half of the successful cases no definite changes were found as regards sperm population, motility duration, or morphology. At present, two possible explanations may be considered. The accessory glands may be stimulated to provide a more wholesome medium for the sperm, e.g. an increased fructose secretion by the seminal vesicles. It is also possible that in some manner, pregnenolone may accelerate or make feasible a more complete or adequate spermatozoal maturation by a direct effect of the steroid upon the seminiferous tubules.

With respect to relative seminal inadequacy, this term is used because it describes fully the situation. As long as there are normal spermatozoa present in the ejaculate, pregnancy is possible even though its probability may be small. In the presence of oligozoospermia, regardless of degree, it is our opinion that the failure of conception will depend to a great extent

upon the fertility potential of the female. Infertility is primarily a problem that involves two people representing a single biologic unit. It follows, then, that it is not a matter of how "good" the male may be or how "normal" the wife is; the problem remains one of the relative fertility of a particular couple.

As a matter of fact, as long as there are apparently normal spermatozoa, regardless of number, attention must also be directed toward improving the fertility potential of the wife to as optimal a level as possible. At the same time, the quality of the seminal ejaculate should also be improved. From a psychosomatic viewpoint, the husband must never be told that the infertility exists because it is his "fault." The tremendous emotional chaos engendered in the tottering of his male ego should deter the doctor from this statement. The Achilles heel in the male ego is any aspersion on his being a "normal" man in every sense of the word. This is especially true since virility and fertility are considered as one and the same by most laymen. Further, since in our experience, several pregnancies have occurred with counts of less than one million per cubic centimeter, the matter of oligozoospermia again becomes a relative one, being dependent upon the fertility potential of the female.

In the clinical management of these couples in whom relative seminal inadequacy (based upon the rather meager and poorly defined standards of our scanty knowledge of the subject) exists, the couple must be made cognizant of the fact that the apparent seminal inadequacy is only a relative one at best, depending primarily upon the fertility potential of the wife. In language that both husband and wife can understand easily and clearly, it must be emphasized that it is the couple who is infertile, and not the husband or the wife; and further, that therapy will involve both partners so as to improve the fertility index of the biologic unit that they represent. In this manner, the husband will co-operate readily and willingly instead of erecting secondary defense mechanisms in which to bury his feeling of inadequacy. Actually, many couples cease trying to have a child of their own, primarily because the husband assumes an air of indifference and refuses to co-operate.

Summary and Conclusions

The factor of relative seminal inadequacy represents one important facet of the problem of human infertility. In its management, the adjuvant use of various steroids was enlisted. The criteria for improvement were twofold: (1) The seminal ejaculate was evaluated from the viewpoint of population, duration of motility, and morphology; and (2) subsequent pregnancy.

The following steroids, in the dosage used, had no discernible effects: progesterone, desoxycorticosterone, and ethinyl testosterone (anhydrohydroxyprogesterone).

Both testosterone propionate intramuscularly and methyl testosterone orally or buccally, in the dosage used, yielded suggestive evidence of producing a decrease of large immature forms of spermatozoa. In these short-

term trials, the effects upon sperm population and motility were rather negligible, except in an occasional instance.

Pregnenolone acetate administered in oil intramuscularly in weekly doses of 50 to 200 mg. yielded the most promising results. Sperm population was increased by 50 per cent or more in 19 out of 40 cases, or 49 per cent. Of the 25 cases of persistent oligozoospermia, 14 patients, or 56 per cent showed this increase in sperm count. On the other hand, when the weekly dose of pregnenolone exceeded 200 mg., a definite drop in sperm count usually occurred.

Motility duration, using eight hours post-ejaculation at room temperature as the end point, was improved 100 per cent or more in 35 per cent of the cases.

Conceptions followed pregnenolone acetate therapy in 18 cases; occurring twice in 4 cases. Of these 22, 8 aborted and 14 delivered normal babies, making the salvage rate 64 per cent.

No correlation was found between improvement in the seminal picture (in only 9 of the 18 successful husbands) and subsequent pregnancy. Thus, it is postulated that pregnenolone acetate may exert a spermatogenotrophic effect which may improve the fertilizing capacity of human spermatozoa.

The results obtained with pregnenolone and perhaps methyl testosterone warrant further clinical study with these and related steroids.

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Discussion

F. X. Gassner: For the past five years we have been interested in the problem of sterility in the male among the domestic animals, particularly that of the bovine species. Our attention has been focused specifically upon functional pathology of the testes and its relationship to the function of the accessory sex organs. The question of the existence of a second testicular hormone, other than estrogen, as was proposed by several workers, has been the subject of extensive investigation in our laboratory following our observation that aqueous fractions of bull testes, which were devoid of either estrogenic or androgenic activity, have a profound effect on the accessory sex organs of the male rat which had been stimulated with testosterone propionate. Similarly, the as yet unexplained in-

hibitory action of such extracts on spontaneous breast tumors in aging female dogs, reported by us in 1947, provided further incentive for continuation of our studies. One of the principal difficulties in this work has been the fact that no critical means of assay of the potency of testes extracts were available. Determination of acid and alkaline phosphatase activity of the accessory sex organs of animals treated with these substances, while indicating certain positive effects of extracts on the functional behavior of these organs, proved to be too cumbersome. Mann and his co-workers reported that the glycolysable sugar contained in seminal plasma, which is the sole energy source available to spermatozoa, is fructose and not glucose as heretofore assumed. The elaboration of fructose by the accessory sex organs appears to be under the control of the androgen produced by the Leydig cell system of the testes. We were able to confirm Mann's findings and extend considerably the investigations of this phenomenon. Upon sexual maturity, fructose is produced in direct proportion to the androgen elaborated by the testes. Following castration, fructose disappears rather quickly, usually within five to seven days, from the seminal plasma and the accessory organs. Implantation of testes or injection of testosterone prevents this loss of fructose. Restitution of accessory gland function with respect to fructose elaboration occurs following treatment with testosterone propionate, but only to the extent of 75 per cent of the normal level. This again indicates that the testes may produce other hormonal factors than testosterone or estrogen, since mixtures of testosterone and estrogen failed to give significantly better results.

We have under investigation several other steroids, particularly certain intermediates of steroid metabolism, such as pregnenolone. In the castrated bull, fructose production returns to a nearly normal level within two weeks after testosterone therapy (100 mg. thrice weekly) while pregnenolone (200 mg. thrice weekly) is similarly effective but only after three to four weeks of treatment.

The testes of the bull can be damaged severely by administration of estrogen (50 mg. estradiol dipropionate thrice weekly). The sperm count drops within seven days and sperm deformities rise to as high as 80 per cent. The large number of juvenile cells present in the ejaculate indicates interference with the spermiogenic phase of spermatogenesis. Serial biopsies taken confirmed this finding. The fructose level remains normal for about three weeks and then suddenly drops, suggesting that the Leydig cell system appears to be considerably more resistant to estrogen damage than is the gametogenic system of the testes. Upon administration of testosterone (100 mg. thrice weekly) the sperm count recovers slowly within three weeks and so does the spermiogenic phase. With pregnenolone a similar recovery occurs, but about a week earlier.

Fructose levels return more rapidly to near normal with testosterone than with pregnenolone, e.g. within two weeks and three weeks, respectively. The unexpected finding that pregnenolone is fructogenic is most interesting since it demonstrates an anabolic effect on the functional behavior of the seminal vesicles of the bull.

These observations are in agreement with the interesting findings reported by Dr. Abarbanel that pregnenolone appears to improve spermatogenesis and fertility in the sterile human male. I am convinced that in the human a similar functional relationship exists between testes, accessory sex organs, and fructose content of seminal plasma. Furthermore, I am hopeful that Dr. Abarbanel will be able to show how this relationship is altered in seminal insufficiency and whether testosterone, or more particularly, pregnenolone, can correct this defect in the human male.

There exists an alarmingly high incidence of breeding failure in domestic animals, particularly in dairy cattle, and it appears certain that any improvement made in diagnosis or therapy would be of considerable economic importance. The selection of fertile breeders is of vital importance, especially since the practice of artificial insemination in the dairy industry has grown to tremendous proportions. The selection of fertile males is based on semen evaluation by methods somewhat more laborious than those employed in human medicine. These methods are not satisfactory, and time-consuming field tests of breeding efficiency have to be made before final certification of a proved sire is accomplished.

The evaluation of seminal fructose not only affords an additional means of estimating semen quality accurately, but it also permits the measure of functional relationships between testes and accessory sex organs. Because the level of fructose in semen plasma or in seminal vesicles or prostate indicates the rate of Leydig cell functions as well as the degree of response of target organs to testicular steroids, there is possible a more accurate diagnosis of testes failure and determination of whether the fault lies with the gametogenic system or the Leydig cell components of the gonad. Consequently, endocrine therapy can be applied more intelligently and effectively. It also appears that with this additional tool the evaluation of steroids or their intermediates with respect to their effects on metabolic functions of target organs and tissues becomes more favorable.

It has been interesting to hear various speakers stress the importance of the relationship of nutrition to hormone action with respect to target-organ response. Recent observations made in our laboratories showed that vitamin B_{12} deficiency associated with a low lysine level in the diet resulted in hypogonadism in chicks. Added lysine caused no improvement, but B_{12} and lysine supplementation resulted in testes development which exceeded that of the normal bird by 100 per cent. Exogenous gonadotrophin was more potent when given with added B_{12} than without. Similar observations have been made by Chow et al. in the rat. Since we also have evidence that vitamin B_{12} encourages amino acid utilization, it may well be that this was responsible for the accelerated target-organ response to hormones. Therefore, it is entirely possible that this phenomenon plays a role in some of the disease processes discussed at this meeting and that it may lend itself to explain in part the discrepancies with respect to therapeutic effects of pregnenolone and other steroids.

I. T. Nathanson: The earlier remarks of Dr. White and those of Dr. Abarbanel on the possible role of thyroid function in the action of steroids prompts me to cite a recent observation. A study of 1500 patients with breast cancer revealed that at least 3 per cent of the group had abnormalities of the thyroid gland at one time or another. This incidence is higher than that usually found in a comparable series of women without breast cancer in the New England region. Recently, we treated a patient with allenolic acid who had advanced breast cancer and obvious myxedema. The disease continued to progress during two and one-half months of treatment. Thyroid extract was then administered in addition to allenolic acid and a definite regression of the lesion occurred in another month. Although this is a single observation and the significance uncertain, I believe that this aspect of the problem of steroid action merits further investigation.

Another point: Is it not possible that pregnenolone might exert its effect on spermatogenesis via the pituitary gland? Experiments by us some years ago revealed that a single injection of either estrogens or androgens in the immature rat produced follicle stimulation in the female and evidence of increased spermatogenic activity in the male. These effects were not seen in hypophysectomized immature animals injected with identical dosages of the same hormones. It would be interesting to try the same experiments with pregnenolone.

H. Selye: In connection with the extratesticular effect of pregnenolone, I just want to point out that in castrate male rats, pregnenolone stimulates the growth of the prostate and of the preputial glands. Such extratesticular effects may also play a role in its beneficial effects in those clinical cases in which no manifest change in sperm count has been seen.

E. C. Reifenstein, Jr.: I would like to ask Dr. Abarbanel if he has any information on the effect of pregnenolone on the testicular biopsies. Does he know anything about possible pathological changes produced by the steroid? I am thinking of the work that Dr. Carl Heller reported last June, in which he showed that the damaging effect of testosterone was a transitory affair. He treated a series of individuals with testosterone propionate for 90 days and controlled the study with a series of biopsies and sperm counts which showed that the subjects were moderately below normal initially. At the end of the treatment period there was marked damage and the count dropped to zero. This damage persisted for a period of six to nine months; then the patients began to recover and after as much as three years were back to normal. In the end, they were better than when they began. I think this emphasizes the long-range nature of steroid studies.

A. R. Abarbanel: As far as testicular biopsy is concerned, I have been rather unimpressed with it from a clinical point of view. From an experimental point of view, I agree fully.

22

Observations on the Effect of Progesterone on Carcinoma of the Cervix*

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The usefulness of hormonal therapy in cancer of the breast and prostate[1, 2] suggested the possible effectiveness of steroid hormones in altering the clinical manifestations of cancer of the cervix uteri. In selecting progesterone as a steroid hormone for clinical trial, we were influenced by certain prior experimental findings with this substance. A lesion entirely comparable to cervical cancer had not been experimentally produced in the primate, but Overholser and Allen[3] and several other observers[4, 5] had shown that prolonged estrogen stimulation in the monkey produced a marked squamous metaplasia of the cervical glandular epithelium. Moreover, Hisaw and Lendrum[4] had shown that such estrogen-induced metaplasia could be entirely prevented by the simultaneous administration of progesterone. Thus, what had been interpreted by many as a pre-cancerous lesion in the cervix could be markedly altered by progesterone.

Lipschutz, Murillo and Vargas[6] demonstrated that estrogen administration would lead to the formation of uterine fibroids in the guinea pig. They also observed that progesterone given along with estrogen would prevent the formation of such estrogen-induced fibromyomata. They referred to this effect as the "antitumorigenic" action of progesterone.

Goodman[7] reported a decrease in size of fibroid tumors in seven patients treated with progesterone. Segaloff, Weed and Parson[8] observed no radiographically demonstrable change in the size of fibroid tumors in 3 cases.

Gardner[9] has shown that the development of cervical cancer in certain strains of mice is dependent upon estrogen administration. Hertz, Larsen and Tullner[10] observed that progesterone could quantitatively

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suppress active tissue proliferation in the genital tract of the estrogen-treated bird.

In view of these experimental findings indicating an antitumorigenic and growth-suppressant action of progesterone, a clinical study of the effect of progesterone* on carcinoma of the cervix was undertaken.

Materials and Methods

Seventeen patients were selected from the Gynecology Clinic of the George Washington University Cancer Service. Their clinical status was determined at the time of their entrance into the study in accordance with the League of Nations classification for cervical cancer.[11] All diagnoses were confirmed by biopsies which were unequivocally interpreted as indicating the presence of malignancy. Only patients who presented clearly visible lesions were selected for this study in order that periodic photographs would permit objective appraisal of the progress of the visible portion of the carcinomatous process.

All patients were given 250 mg. progesterone in 5 cc. of oil intramuscularly daily for varying periods as indicated in Table I.

The following observations were made on all patients just prior to and at frequent intervals during the course of progesterone administration:

1. Photographs of the cervix taken with the aid of a Coreco camera under conditions of fixed illumination and magnification.

2. Daily urinary excretion of sodium pregnanediol glycuronidate as determined by the method of Sommerville et al.[12]

3. Weekly pelvic examinations noting the visible and palpable changes in the cervix and parametria with special emphasis on the color and size of the lesion, its mobility and friability and the presence or absence of active bleeding.

4. Weekly appraisal of the patient's general clinical status with respect to (a) vaginal bleeding or discharge, (b) pain, (c) feeling of well being, (d) appetite, (e) urinary function, (f) bowel function, (g) body weight and (h) possible local or systemic effects of the progesterone.

Immediately after the completion of the progesterone study 14 of these patients were afforded definitive surgical management as indicated.† One patient who presented far advanced disease (#2) was continued on progesterone until she died and one patient (#1) discontinued attendance at the clinic several months following her 153-day course of progesterone.

The age, color, clinical classification, duration of progesterone treatment and other pertinent features of each case are set forth in Table I.

* Progesterone used in these studies was provided by the Schering Corporation through the courtesy of Dr. E. L. Henderson.

† We will subsequently present a detailed report on the surgical aspects of these studies.

TABLE I

CANCER OF CERVIX UNDER PROGESTERONE ADMINISTRATION

Case No. Age and Color	*Description*	*Biopsy*	*Previous Treatment*	*Progesterone Administered*	*Urinary Pregnandiol Recovery*	*Clinical Response*	*Surgery*	*Surgical Specimen*
1 77 C	Recurrent Stump Stage III	1947 Epid. Ca. gr. 3 March, 1949 Residual Ca. August, 1949 Epid. Ca. gr. 2	1925 Subtotal 1947 – 48 Full X-ray and Radium	38.25 Gm. in 153 days	4%	Discharge decreased Extension delayed 3 mo.	None	None
2 43 W	Recurrence 4 mo. after Wertheim Recurrent Stage IV	August, 1949 Epid. Ca. gr. 3	1948 – 49 Radium Jan. 1949 X-ray May 1949 Wertheim	34.5 Gm. in 138 days	10%	Bleeding decreased Softening Leg pain lessened	None	None Autopsy: Extension to femoral plexus
3 65 W	Ulcerating Stage II	Epid. Ca. gr. 2	None	16.25 Gm. in 65 days	Not Done	Cessation of bleeding Epithelization	Radical with Nodes	Epid. Ca. gr. 2 Atrophy- endometrium
4 56 W	Bilat. ooph. 1926 Stage III	Epid. Ca. gr. 2	None	28.85 Gm. in 113 days	10%	Bleeding ceased Relaxation Epithelization	Radical with Nodes Cystectomy Bilat. Uret. Transplant	Epid. Ca. gr. 3 with Extension to Corpus 3 of 18 nodes positive. Atrophy- endometrium
5 54 W	Asymptomatic Erosion Stage I	Epid. Ca. gr. 3	None	1.5 Gm. in 10 days	13%	Epithelization Softening	Radical with Nodes	Epid. Ca.—in situ

6 36 W	Ovarian cyst Erosion Late Stage I	Epid. Ca. gr. 2	None	2.0 Gm. in 10 days	Not Done	Epithelization Softening	Radical with Nodes	Epid. Ca., gr. 2 Pseudomucinous cystadenoma—ovary
7 60 W	Asymptomatic Small erosion Early Stage I	Ca. in situ	None	2 Courses 18.5 Gm. in 74 doses over 103 days	8.4%	Cervix Less Vascular	Radical with Nodes	Chronic Cervicitis Endometrial polyp Endometriosis of uterus
8 50 C	Crater Stage I	Epid. Ca. gr. 2	Radium 1933 for Menor- rhagia	6.0 Gm. in 28 days	8.5%	Crater filling Epithelization Softening	Radical with Nodes	Epid. Ca., gr. 2 plus radiation effect
9 62 W	Ulcer Residual from Stage I	1948, Epid. Ca. 1949, Epid. Ca. gr. 3	1948 X-ray and Radium full course	1.2 Gm. in 12 days	Not Done	Epithelization Softening	Radical with Nodes Cystectomy Bilat. Uret. Transplant	Epid. Ca. Vagina Radiation effect Adenomatous polyp of endometrium
10 58 C	Crater Stage IV	Epid. Ca. gr. 3	1948 subtotal	3.15 Gm. in 21 days	6%	Bleeding ceased Softening Deceased in P.O. uremia	Stump with Nodes Cystectomy Vaginectomy Uret. Transplant	Epid. Ca., gr. 3 Cystitis Hydrosalpinx
11 31 W	Sterility Asymptomatic Cystic Cx Early Stage I	Sq. Ca. gr. 2	None	7.25 Gm. in 31 days	Not Done	Cervix regained normal appearance	Radical with Nodes	Ca. in situ Prolif. endometrium hemorrhagic corpus-luteum

Key to Abbreviations Stage—indicates League of Nations classification of the disease P.O.—postoperative

TABLE I—*(Concluded)*

Case No. Age and Color	*Description*	*Biopsy*	*Previous Treatment*	*Progesterone Administered*	*Urinary Pregnandiol Recovery*	*Clinical Response*	*Surgery*	*Surgical Specimen*
12 61 C	Stage III	Epid. Ca. gr. 2	None	16 Gm. in 64 days	13%	Discharge diminished Bleeding ceased Deceased – P.O. Acidosis + abscess	Radical with Nodes Cystectomy Bilat. Uret. Transplant	Epid. Ca., gr. 2 Autopsy: Pelvic abscess
13 65 C	Ulcer Stage III	Epid. Ca. gr. 3	None	12.5 Gm. in 50 days	5.5%	Discharge and Bleeding Ceased Epithelization Softening Pulmonary Infarcts	Radical with Nodes Vena Caval Ligation	Epid. Ca., gr. 2
14 53 C	Crater Stump Stage III	Epid. Ca. gr. 2	Subtotal at age 17	7.425 Gm. in 61 days	19%	Bleeding Ceased Crater healed Pain less; then ceased 6 mos. P.O. Well	Radical Stump plus Nodes Cystectomy Vaginectomy Uret. Transplant	Epid. Ca., gr. 2
15 36 C	Bleeding Erosion Stump Stage I	1948 Epithelial Hyperplasia 1950 Ca. in situ	Subtotal at 34	7.25 Gm. in 31 days	Not Done	Bleeding Ceased Lesion Cleaner	Radical with Nodes	Epid. Ca., gr. 1
16 35 C	Vag. disch.—2 mo. Exophitic Stage II	Epid. Ca. gr. 3	None	16.25 Gm. in 65 days	Not Done	Normal menst. under prog. 2 x Epithelization Softening	Radical plus Nodes	Epid. Ca., gr. 3
17 53 C	Erosion Stage III	Epid. Ca. gr. 3	None	42.5 Gm. in 149 doses in 170 days	3.3%	Bleeding Stopped Epithelization	None	None

Key to Abbreviations Stage—indicates League of Nations classification of the disease P.O.—postoperative

Results and Discussion

In such a widely varying and yet limited body of clinical material it is difficult to make general observations. Nevertheless, certain changes in the clinical and morphological features of the lesions in the progesterone treated patients occurred with sufficient frequency to warrant description.

In eleven of the 17 treated patients visible and palpable evidence of regressive alteration of the tumor mass could be demonstrated. This consisted of (a) distinct reduction in size of the visible portion of the cancer as well as reduction of the palpable extent of the mass, (b) reduction in vascularity and friability of the visible lesion with a clearly demonstrable epithelization of previously raw surfaces and (c) markedly increased pli-

FIG. 1. From Case 11 (see Table I).
(A) initial appearance of lesion, (B) after 31 days on progesterone.

ability of the previously rigid and infiltrated parametria. Fig. 1 illustrates the regression of an early lesion under the influence of progesterone in case #11. Fig. 2 (case #16) illustrates the reduction in size of the largest lesion we have studied. Fig. 3 (case #13) represents a less marked but more frequently observed type of regressive change.

In 10 cases there was associated with this type of gross change a reduction in, or complete cessation of, vaginal bleeding and discharge.

Routine histopathological study of the biopsy and surgical specimens has not indicated the morphological basis for the observed changes. A subsequent report will present a detailed histopathological study of serial blocks of the material from all of these cases.

Only one of the 17 patients (#1) showed active progression of the carcinomatous process while under progesterone administration. The six patients whose lesions failed to show clearly demonstrable regressive

changes showed minor alterations in size and vascularity of insufficient degree to be convincing to all clinical observers concerned. Nevertheless, none of the lesions under study appeared to be accelerated by progesterone.

Only one untoward side-reaction was observed. This was in a patient who proved allergic to the vegetable oil used as a vehicle for the progesterone. Injections were discontinued after 3 days and this patient is therefore not included in the present series. Otherwise, the progesterone was well tolerated in all respects. However, the necessity for daily adminis-

FIG. 2. From Case 16 (see Table I).
(A) initial appearance of lesion, (B) after 65 days on progesterone.

FIG. 3. From Case 13 (see Table I).
(A) initial appearance of lesion, (B) after 50 days on progesterone.

tration of 5 cc. of oil intramuscularly in some cases led to difficulty in varying the site of injection and this made the patient somewhat uncomfortable. It is noteworthy that three patients who were menstruating regularly before treatment continued to menstruate during the course of such massive dosage with progesterone (see Table I).

The percentile recovery of the total dose of administered progesterone in the form of urinary pregnandiol is tabulated for 11 cases in Table I. These figures are in good agreement with comparable data reported by Sommerville and Marrian[13] for normal adult females. This indicates that by this admittedly crude measure, the patient suffering from cervical carcinoma metabolizes progesterone in a grossly normal manner.

We do not consider the regressive changes observed to be sufficient to indicate the use of progesterone as a therapeutic agent in carcinoma of the cervix. Nevertheless, these effects do indicate that cervical carcinoma can be substantially altered by steroid hormone administration. In some instances these changes have been somewhat comparable to the hormone-induced regressions seen in breast carcinoma under estrogen therapy.[1] Such phenomena constitute a chemotherapeutic lead, indicating the general class of compound which may in time prove efficacious as a truly therapeutic agent. In this connection, Lipschutz[14] has surveyed a large series of steroids for "antitumorigenic action." He concludes that antitumorigenic action is not related to biological hormonal activity. He adds that "there is place for the hope that antitumorigenic steroids having no other pharmacological action may be obtained by chemical synthesis."

Our limited observations reviewed in the light of these experimental findings will serve as a basis for further study of the effect of this family of steroid compounds upon the clinical manifestations of cervical cancer.

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Discussion

Discussion on this paper follows Chapter 24.

23

Newer Steroids in the Treatment of Advanced Mammary Carcinoma*

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The position of conventional steroid hormone therapy in the treatment of advanced inoperable breast carcinoma of the female is well established, and statistics on the incidence of improvement are now considered fairly valid. Androgens will produce approximately 20 per cent objective improvement in osseous disease and approximately 30 per cent improvement in soft-part metastases. Estrogens in the appropriate age group, i.e. women 10 years or more than 10 years postmenopausal, will cause an approximately 30 per cent osseous regression and 50 per cent soft-part regression.[1] These are small percentages of temporary gain, but they are of value to advanced-cancer groups. Most of the investigators in the field, however, deplore the fact that a higher rate has not been achieved and that one is faced with various undesirable side-effects from the use of the standard male and female hormones with their considerable sexogenic activity. The orthodox compounds in these two groups have also shown profound effects on body systems other than the ones under discussion, particularly the hematopoietic system,[2] salt and water metabolism,[3] and protein metabolism.[4] The question has been raised, therefore, as to whether it would be possible to develop steroid hormones which could have a good carcinolytic effect without some of these undesirable side-effects.

During the past year, two investigators reported on the effects of methyl androstenediol. Gordan and co-workers[5] reported on the protein anabolic effect of this compound, and felt that there was diminished sexual stimulation. Homburger et al.[6] reported on the carcinolytic and metabolic effect of this compound on metastatic breast carcinoma.

The group at Memorial Cancer Center has had on clinical trial

* This work has been aided by a grant from the National Cancer Institute of the United States Public Health Service.

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several newer steroid compounds. This preliminary work is now presented in the interest of stimulating further studies so that better preparations may be made available both for their primary effects and for minimizing the undesirable side-effects.

Six cases of advanced inoperable mammary carcinoma were treated with dihydrotestosterone,* an androgen compound, on a dosage schedule of 100 mg. per day, six days a week, intramuscularly. Five of these patients were in the androgen age group, the other was more than 10 years post-menopausal and had previously shown a good response to estrogen therapy. All these cases were considered progressive and one in particular had shown hypercalcemia on testosterone propionate. All six patients have shown distinct symptomatic improvement, with relief of nausea, vomiting, anorexia, and weakness, where present. Protein anabolism was evidenced by weight gain without edema formation.

Two of these patients with osseous metastases, also having elevated blood calcium and phosphorus levels, showed a distinct drop in the blood concentrations of both factors after 1200 and 3700 mg. of dihydrotestosterone, respectively.

A third patient, who had right lower quadrant intra-abdominal recurrence, showed a diminution in the size of the mass by one-third. There was healing of a breast ulceration after 2.9 gm. in another of the patients. Two of the patients demonstrated some hirsutism after four weeks of therapy, but this was minimal in comparison to the effects of testosterone and testosterone propionate. There was no libido stimulation, and only one patient developed some slight increase of unilateral lower extremity edema.

Two patients were treated with the acetate derivative* of dihydrotestosterone on the same dosage schedule. One of these patients, with several areas of osseous metastases, demonstrated an alkaline phosphatase flare from 12.4 to 40.1 Bodansky units after only 500 mg. of therapy. The alkaline phosphatase gradually dropped when the patient was admitted to the hospital and therapy stopped. After the patient had been off therapy for several weeks, a shock-like episode occurred which was diagnosed as coronary thrombosis and we were unable to repeat the experiment under more controlled conditions. In the second patient treated with the acetate derivative, there was extensive cutaneous disease, some parts of which were treated with the Phillips machine as an adjunct research project, with careful shielding to prevent scattering to adjacent areas. After less than one month of androgen therapy, for a total dose of 2500 mg. there was healing of ulcerations and shrinking and flattening of cutaneous nodules in the areas influenced by the hormone only.

Five patients have been treated orally with methyl androstenediol† on a dosage schedule of 100 mg. daily. Therapy in one patient was terminated after one week because of increased respiratory difficulty. Two

* Dihydrotestosterone and dihydrotestosterone acetate were supplied by Chemical Specialties Company, Inc., and Syntex, S. A.

† Methyl androstenediol was supplied by the Schering Corporation.

of the others with osseous disease have demonstrated responses which would suggest that this compound is capable of affecting bone metabolism. Of these, one patient, 41 years of age, two years postroentgen castration, showed an alkaline phosphatase rise from 12.0 to 18.7 B.U. after six weeks of therapy, and considerable symptomatic improvement was reported. The other patient, who had previously been on testosterone cyclopentylpropionate, 30 mg. three times a week, which was terminated at the patient's request because of increased libido, was started on methyl androstenediol one month late. The demonstration of regression of disease in the lumbar spine, ilium, and skull, two weeks after the institution of this second compound, was felt to be a continuation of the previous testosterone cyclopentylpropionate effect. Furthermore, after this patient had received 8200 mg. of methyl androstenediol in a period of approximately nine weeks, she suddenly developed a hypercalcemia of 16.3 mg. with its accompanying clinical syndrome, and in addition complained of more bone pain. With cessation of treatment, there has been a slow drop in the blood calcium level and improvement in symptoms. The other two patients have been on therapy for only two months. They demonstrate nothing but symptomatic improvement to date.

It is felt that the significance of the changes in alkaline phosphatase and calcium, as demonstrated in two of the patients in this group of five, suggests that calcium metabolism can be definitely affected. It is interesting to note, also, that no libido stimulation has been found to date and that there has been only slight hirsutism. The hirsutism seems to be even less than that seen with dihydrotestosterone. It is realized that the presentation of a small group of patients like this is of little statistical value, but we are in an exploratory mood, searching for newer compounds.

With all this discussion of the effects of those androgens which are less sexogenic, one must not lose sight of an important question. Is the diminished androgenic effect simply a reflexion of the fact that on an overall basis these compounds are less potent? If we give them in large enough doses, would we produce the same degree of masculinization and objective improvement in carcinoma metastases?

In April 1949, our group was asked by the Committee on Research of the A.M.A. to run a series of appropriate cases on schedule D, testosterone, 200 mg. three times per week, which on a milligram basis, is comparable to the two compounds on discussion today. A review of the 30 patients in this group reveals that only one patient with osseous metastases and two patients with soft tissue involvement showed objective improvement. Salt and water retention, hirsutism, voice changes, and increased libido were much more troublesome, and the usual hematopoietic stimulation seen with testosterone, 100 mg. three times per week, did not seem to occur. No attempt has been made at present to increase the dosage of the new compounds to the point where severe side-effects would be produced, because it appears at the present time that, in the dosage employed, they are capable of evoking a carcinolytic effect.

The aforementioned data suggest the possibility that some steroid

hormones can have a satisfactory effect against carcinoma with less undesirable side-effects than seen with those compounds previously employed.

It must again be emphasized that this is at best a preliminary report on these newer compounds and that considerably more work is necessary before their true evaluation can be established.

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Discussion

Discussion on this paper follows Chapter 24.

24

Screening of Steroids and Allied Compounds in Neoplastic Disease*†

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The natural course of certain neoplastic diseases as well as other growth processes may be governed by steroid hormones. This suggests that one insight into the basic mechanisms of growth might be gained by a comprehensive study of the relative effects of natural and synthetic steroid compounds possessing different types and degrees of functional activity and chemical configurations. It is visualized that some common denominator might be unearthed which would provide essential information concerning further avenues to the study of endocrine-cancer relationships. Accordingly, we have designed a program to test this hypothesis. Among the studies performed on patients with neoplastic diseases and suitable control subjects submitted to steroid therapy are: (1) Clinical evaluation of the response of the tumor, (2) attempts to correlate systemic and other effects with the changes in the tumor, (3) metabolic alterations, and (4) determination of excretion patterns of urinary steroids before, during, and after hormonal administration.

In order to evaluate properly and correlate these various phases of the problem, it is essential that baselines be established which can be used as reference standards. Thus, we have chosen as reference standards the natural history of the disease and those steroids and laboratory techniques that are best understood. It is of great interest and perhaps significant that thus far only four types of neoplastic diseases appear to be susceptible to steroid therapy. These are cancer of the breast, prostate gland, and

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cervix, and neoplasms of lymphogenous origin. Breast cancer was chosen primarily for the screening procedures, because the manifestations of this tumor are usually accessible for systematic clinical, radiologic, and histologic studies. Since breast cancer commonly metastasizes to the skeletal structures, an opportunity is provided for performing metabolic studies that can be compared to those in patients with osseous involvement from other types of neoplasms and bone disease of other origin. Furthermore, sufficient information is available on the effects of the common steroids in breast cancer to establish an adequate baseline for evaluation of new compounds. Prostatic cancer is also exceedingly valuable as a test object for the reasons stated above. In addition, the abnormalities of the serum acid and alkaline phosphatase levels frequently seen in this disease are of inestimable aid in estimating the potential carcinolytic properties of a compound before objective responses in the tumor can be detected. Cancer of the prostate gland is highly susceptible, usually more so than breast cancer, to alterations of the hormonal balance of an individual. However, this unusual responsiveness may mask subtle differences among the variety of compounds that are subjected to testing. Consequently we have used prostatic cancer essentially for preliminary trial. Some of our observations on the effect of various steroid hormones and allied compounds in prostatic and other neoplasms will be discussed. The main body of data will be concerned with breast cancer. It should be emphasized that care in the selection of patients was exercised so that an individual series was comparable to another.

Estrogenic Hormones

Clinical Observations. Ten estrogenic hormones have been tested in patients with breast cancer. These include (1) compounds that are closely allied chemically but which vary considerably in their estrogenic activity on a dose basis, and (2) compounds that have a different chemical structure and also varying degrees of biologic activity. Diethylstilbestrol was used as a reference standard and all compounds were administered orally. Since the large majority of the patients were ambulatory when therapy was instituted, it was essential to ascertain that the compounds were taken in sufficient amounts. Consequently, when possible, vaginal smears were taken from each patient at every visit. Under other circumstances, stigmas characteristic of estrogens were carefully investigated. In this way, there is reasonable assurance that the patients included in the analyses had a sufficient trial of a compound to be included in any given series. The types of compounds, average dosage levels, and the effects on the soft tissue manifestations of breast cancer, since these are most characteristically influenced by estrogens, are shown in Table I. All patients in these groups were treated for a minimum of two months, since, as can be seen from Table II, this period is critical for an evaluation of the effects of an estrogenic hormone. The great majority of patients were treated with diethylstilbestrol, since we were most interested in establishing a baseline with a single active hormone at the outset of these studies.

TABLE I

EFFECT OF VARIOUS ESTROGENS ON SOFT TISSUE LESIONS OF BREAST CANCER

Compound	*Number of Cases*	*Average Daily Dose, Mg.*	*Per Cent Regression*
Diethylstilbestrol	145	15	41.0
Dimethyl ether of diethylstilbestrol	21	20–30	48.0
Monomethyl ether of diethylstilbestrol	15	20–30	47.0
Dienestrol			
low dose	7	1–3	14.0
high dose	9	30	44.0
Hexestrol	7	24	57.0
Benzestrol	7	30	30.0
Ethinyl estradiol	28	3–4.5	54.0
Sodium estrone sulfate (Premarin)	21	20–30	48.0
Methyl bisdehydrodoisynolic acid	13	20–30	23.0
Allenolic acid	4	7.5–15	25.0
Tripara-anisylchlorethylene	6	24–48	17.0

The number of patients treated with the other compounds is considerably smaller, but in some groups a sufficient number of patients has been observed for relative comparison. On the basis of the present studies even in those series where the number of patients is very small, it appears that any compound that possesses any degree of estrogenic activity, regardless of chemical structure, has an effect on the soft tissue lesions of

TABLE II

ESTROGENS

DURATION OF TREATMENT TO INITIAL RESPONSE

Months	*Number of Patients**	*Cumulative Percentage*
0.5	2	1.6
1.0	63	50.3
1.5	17	63.6
2.0	35	90.7
3.0	11	99.2
4.0	1	100.0
Total	129	

* Includes only patients responding to estrogen therapy.

breast cancer. As regards the percentage response, hidden factors other than the total number of patients and dosage in a series may materially influence the effect so it is not possible to single out any one compound as the most efficacious. This is true in spite of every attempt to make each series comparable as to menopausal status and age of the patient, site of the lesion, and the general physical condition of the patient.

Dosage is another important factor that must be considered in a study of this kind. An attempt was made to use dosages of the hormones that were considerably greater than those usually necessary to control menopausal symptoms and to produce cornification of the vaginal cells. These dosage levels were selected on the premise that individual susceptibilities could be excluded. Even under these circumstances, which were based on comparative studies of the estrous capacities of the compounds in the animal, it was obvious that several of the compounds were

TABLE III

COMMON SIDE-EFFECTS OF DIETHYLSTILBESTROL

Side-Effects	*Total Number of Cases Treated*	*Number of Cases with Undesirable Reactions*	*Per Cent*
Gastrointestinal	230	133	58
Genitourinary	149	41	28
Uterine bleeding	186	63	33
Pigmentation	188	150	80
Edema	193	65	34

not as active as anticipated in the human being. Accordingly, the dosage levels of these compounds were increased considerably in order to provide a statistical validity to the experimental observations. It is also clear that variations in the relative responsiveness of the cancers treated are intimately involved. But it should also be considered that cancers not responding to apparently appropriate dosages may occasionally react favorably to higher dosages. Of further interest are our observations that in some cases a second compound may be effective when one usually active estrogen has failed to produce any effect. This observation suggests the remote possibility that the chemical structure of the compound in a particular patient may be as important as the relative biologic activity. However, this potentiality must be tempered by the fact that the compounds more commonly utilized have been interchanged without pointing to one or another as unusually effective.

Side-Effects. At the dosage levels utilized, every compound tested has produced the common side-effects attributable to estrogenic hormones (Tables III and IV). While some appear to be more or less productive of certain of these effects, it must be emphasized that individual suscepti-

bilities, dosage levels, and other factors must be considered. It is of particular interest that a hormone with a distressing effect on one individual may be well tolerated by another. Substitution of another compound not tolerated by other patients may be entirely acceptable.

Metabolic Studies. Metabolic studies are seldom of significance in the absence of osseous metastases. A beneficial shift after estrogen therap may occur when patients are in negative nitrogen balance. When osseous metastases are present, metabolic and hematologic defects may be corrected. Fig. 1 illustrates an example of this, although in this case the initial effects were deleterious.

TABLE IV

SIDE-EFFECTS OF OTHER ESTROGENS*

Compound	*Cases*	*Gastro-intestinal*	*Genito-urinary*	*Uterine Bleeding*	*Pigmen-tation*	*Edema*
Monomethyl ether	17	7	2	4	7	5
Dimethyl ether	25	5	1	7	14	5
Dienestrol	21	4	5	5	12	5
Hexestrol	7	1	5	3	5	4
Benzestrol	7	3	1	3	3	2
Ethinyl estradiol	36	19	12	6	22	12
Sodium estrone sulfate	28	12	4	8	5	3
Methyl bisdehydro-doisynolic acid	16	3	5	6	5	2
Allenolic acid	5	0	0	1	0	0
Tripara-anisyl-chlorethylene	7	2	0	1	0	0

* Number of cases under each item.

Steroid Excretion Studies. The administration of estrogenic compounds to breast cancer patients has not produced any significant variations to date in the steroid excretion patterns. Because of the large number of determinations of the various steroid complexes that are necessary, it has not been possible to accumulate a significant number of patients.

Androgens

Ten androgenic or closely allied compounds have been screened in breast cancer. The large majority of patients have been treated with testosterone or its active esters. The remaining patients treated with the other compounds are too few in each series to arrive at any conclusions regarding their effectiveness on a clinical and metabolic basis. As with estrogenic hormones, compounds are being sought that will produce the desired changes without the frequently troublesome side-effects. Testos-

FIG. 1. The daily intake is charted from the 0 line downward, and the average daily excretion from the bottom line upward. A negative balance is therefore indicated by extension of the column above the line; and a positive balance, by a clear area below the 0 line.

TABLE V

ANDROGENIC COMPOUNDS

Compound	*Average Dosage Mg.*
Testosterone	50–100 t.i.w.*
Testosterone propionate	50–100 t.i.w.
Testosterone cyclopentylpropionate	30– 60 t.i.w.
Methyltestosterone	30– 60 daily†
Dehydroepiandrosterone	50–100 t.i.w.
17-Methylandrostenediol	100 t.i.w.
17-Vinyl testosterone	100 t.i.w.
Androstenediol	100 t.i.w.
Androstenedione	100 t.i.w.
Testolactone	100 t.i.w.

* t.i.w. Three times weekly—intramuscular injection.
† Oral administration.

terone propionate has been used as a reference standard. The compounds tested and the average dosages are listed in Table V. Since androgens primarily affect osseous metastases and offer considerable subjective relief from symptoms, these two responses were used as a means of detecting a clinical effect. Table VI shows the effects of two commonly employed androgens. There is little difference in the effect of testosterone propionate and testosterone cyclopentylpropionate, although the latter is apparently more slowly absorbed. All patients were treated for a minimal period of two months since, as with the estrogens, this time interval is essential to determine adequately the efficacy of the compound (Table VII). The data indicate, thus far, that the more biologically active compounds produce more consistent beneficial effects. However, this impression may have to be modified when a sufficient number of patients in any one series can be treated with the other compounds. Dosage levels and subtle factors again must be given strong consideration in testing these agents. These should be better understood when more patients are studied.

TABLE VI

EFFECT OF TESTOSTERONE ON OSSEOUS METASTASES OF BREAST CANCER

Compound	*Number of Cases*	*Per Cent Subjective*	*Per Cent Objective*
Testosterone propionate	92	81.5	23.4
Testosterone cyclopentyl-propionate	10	60.0	20.0

Side-Effects. Testosterone, testosterone propionate, testosterone cyclopentylpropionate, and methyltestosterone have all produced the usual side-effects attributed to androgens. These have not been encountered to any extent with the remaining compounds tested in small series of cases. These side-effects, even with the more biologically active compounds, vary considerably among patients so that other factors undoubtedly are operating.

Metabolic Studies. Metabolic studies with the truly androgenic compounds have been conducted only on patients with osseous metastases. If the patient responded satisfactorily, the usual metabolic and hematologic abnormalities were partially if not completely corrected. However, in some instances favorable metabolic shifts occurred without an objective response in the lesion, and the reverse situation has also been seen.

TABLE VII

TESTOSTERONE

DURATION OF TREATMENT TO INITIAL RESPONSE

Months	*Number of Patients**	*Cumulative Percentage*
1.0	16	43.2
1.5	7	62.2
2.0	12	*94.6*
3.0	1	97.3
4.0	1	100.0
Total	37	

* Includes only patients responding to testosterone therapy.

Steroid Excretion Studies. The steroid excretion patterns obtained in 11 patients are summarized in Fig. 2. All 11 patients received testosterone and three received dehydroepiandrosterone as well.

The urinary ketosteroid excretion rose in all patients to values ranging from 2 to 13 times the normal level. This was true both in the testosterone and in the dehydroepiandrosterone experiments.

The pattern of response of the nonketonic steroid alcohols was irregular. Two of the six patients with cancer of the breast showed an increased excretion of nonketonic steroid alcohols while under testosterone therapy; the remaining four excreted significantly reduced quantities of nonketonic alcohols. Both patients with breast cancer who received dehydroepiandrosterone excreted increased amounts of nonketonic alcohols, as did the one patient with cancer of the prostate. Three of the four patients with rheumatoid arthritis who were treated with testosterone excreted smaller quantities of nonketonic alcohols while under treatment, and the fourth showed no significant change. In contrast with the cancer patients who received dehydroepiandrosterone, the one arthritic patient showed an increased nonketonic alcohol excretion.

FIG. 2. Steroid excretion patterns during androgen treatment.

FIG. 3. Steroid excretion patterns as affected by androgen therapy.

Total estrogen assays were carried out in seven of the eleven patients. Both cancer patients in whom estrogen excretion was studied showed a significantly increased excretion while under dehydroepiandrosterone or testosterone therapy. The one patient with rheumatoid arthritis who received both hormones failed to excrete additional estrogen. Two of the three other arthritic patients did, however, have a significantly higher estrogen excretion while under testosterone therapy.

FIG. 4. Steroid excretion patterns as affected by androgen therapy.

More systematic studies were carried out in the cases of R. C. (female, age 69, cancer of the breast), R. MacD. (female, age 35, cancer of the breast, castrated by x-ray), and E. A. (female, age 68, rheumatoid arthritis). In all three patients the administration of either dehydroepiandro-

FIG. 5. Steroid excretion patterns during androgen therapy.

FIG. 6. Nonketonic alcohol excretion during androgen therapy.

sterone or testosterone was followed by a striking rise in the excretion of both ketosteroids and *total* steroid alcohols (Figs. 3, 4, 5), and a fall to or below the control levels when the administration of the hormone was terminated.

When the daily excretion of nonketonic alcohols of these three patients is plotted in terms of percentage change from the pretreatment control level, striking variations in excretion patterns become apparent.

FIG. 7. Effect of androgen therapy on nonketonic alcohol excretion.

In patient R. C. during the period of dehydroepiandrosterone administration, there was an initial fall in nonketonic alcohol excretion, followed by an increased excretion of this fraction. When testosterone was substituted for dehydroepiandrosterone, the nonketonic alcohols promptly fell to extremely low levels (Fig. 6). This picture may be contrasted with that shown by patient R. MacD. (Fig. 7) in whom the administration of both dehydroepiandrosterone and testosterone caused a markedly increased excretion of nonketonic steroid alcohols. A further variation is

shown by patient E. A. (Fig. 8) whose nonketonic alcohol excretion is markedly depressed both by dehydroepiandrosterone and by testosterone.

The daily excretion of estrogens was followed in the same three patients (Figs. 9, 10, 11). Although there were some day to day variations, the average daily excretion of estrogen during the periods of dehydroepiandrosterone administration was significantly higher than during the control periods in the two cancer patients, and was unchanged in the patient with rheumatoid arthritis. Testosterone administration caused a still further rise in estrogen excretion in the two cancer patients and no change in the patient with rheumatoid arthritis.

FIG. 8. Effect of androgen therapy on nonketonic alcohol excretion.

In the cases of the two cancer patients, R. C. and R. MacD., sufficient material was accumulated to characterize the estrogens by countercurrent distribution. During the control period, patient R. C. did not excrete detectable amounts of the three major urinary estrogens, estrone, estradiol-17β and estriol, although the excretion of fluorogenic phenols was 51 μg. per day. When dehydroepiandrosterone was administered, the titer of fluorogenic phenols rose and recognizable amounts of estrone and estriol were excreted. A still further increase in both the total value and

FIG. 9. Estrogen excretion as affected by androgen therapy.

FIG. 10. Estrogen excretion as affected by dehydroepiandrosterone (DHA) or testosterone propionate (TP) administration.

the values for estrone and estriol was observed during the period of testosterone administration.

During the control period, patient R. MacD. excreted fluorogenic phenols at about the same level as patient R. C. and again no significant amounts of the three known estrogens. During the period of dehydroepiandrosterone administration, estriol was identified, although estrone and estradiol were not present in detectable amounts. However, when testosterone was given, all three compounds were excreted in significant amounts.

In patient E. A. there was no significant change in the excretion of "total estrogen," hence no attempt was made to investigate the phenolic fraction by countercurrent distribution.

FIG. 11. Estrogen excretion as affected by dehydroepiandrosterone (DHA) or testosterone propionate (TP) administration.

While no generalizations may be made from the study of a small series of patients, it is evident from the data presented that striking variations in steroid excretion patterns are obtained when the daily excretion of several classes of steroids is followed.

Increased excretion of ketosteroids has been the rule after testosterone and dehydroepiandrosterone administration. The variations in the excretion of nonketonic alcohols after the administration of dehydroepiandrosterone may be the resultant of two phenomena: (1) the inhibition of the endogenous production of the precursors of the urinary nonketonic alcohols and (2) varying metabolic pathways for the degradation of dehydroepiandrosterone.

The question of origin of the estrogen excreted after androgen ad-

ministration can be discussed only speculatively. It is conceivable that the administration of dehydroepiandrosterone or testosterone in the proper metabolic environment stimulates the endogenous production of small quantities of estrogen. On the other hand, the conversion of androgen to estrogen is also an attractive hypothesis. The increased excretion of estrogens after testosterone may be a partial explanation of the effect sometimes observed on the soft tissue lesions of breast cancer.

Progesterone and Adrenocortical Compounds

Eight patients with breast cancer have been treated with progesterone in dosages ranging from 100 to 200 mg. daily by intramuscular injection. No significant regression was observed in any of the patients. A few suggestive responses warrant further testing of this and allied compounds.

Several patients with breast cancer are currently under treatment with cortisone. One of these patients has severe hemolytic anemia which has responded favorably, but no effect on the tumor has occurred. It is planned to treat other patients with other adrenocortical compounds when enough material can be obtained for adequate testing. We have observed the effects of cortisone on malignant lymphatic diseases reported by others. As indicated above, before a significant evaluation can be made, a statistically adequate number of patients must be submitted to trial.

Metabolic Effects and Steroid Excretion. The data are as yet incomplete on the patients treated with progesterone and cortisone.

Other Cancers

Prostatic Cancer. A number of the compounds listed above have been tested in prostatic cancer. Of the estrogens used, all have produced responses that are difficult to distinguish from one another. Testosterone has been used in several cases of prostatic cancer and in each instance there was a significant rise in the serum acid phosphatase levels, which promptly fell after androgen therapy was terminated. No significant clinical effect was seen in any of the patients, although after the hormone was discontinued, one individual appeared to be temporarily relieved of symptoms that existed prior to and during androgen therapy. 17-Methylandrostenediol was tested in two patients with prostatic cancer. One had normal acid and alkaline phosphatase values and there was no effect on these values over a four-week period of therapy. The other had elevated acid and alkaline phosphatase levels. The acid phosphatase levels rose and the alkaline phosphatase levels fell during a three-week period of treatment. On this basis, 17-methylandrostenediol simulates active androgenic compounds. Neither of these two patients derived any clinical benefit from 17-methylandrostenediol.

Progesterone was used in one patient with high serum acid and alkaline phosphatase levels. The acid phosphatase levels declined to significantly lower levels during a period of three weeks and remained at that level. The alkaline phosphatase values rose to much higher levels.

In spite of this, the patient experienced little relief of symptoms and there was no noticeable effect on the tumor.

Cancer of the cervix has been treated with estrogens, androgens, and progesterone. In our hands, no obvious effect has been noted although the results reported at this conference by Hertz clearly indicate that progesterone needs extensive trial.

Estrogens and androgens have been tested in patients with various types of lymphomatous disease, but no specific effect on the tumor was noted. Several patients, however, were improved subjectively.

Relative Effects of Estrogens and Androgens. The comparative effects of estrogens and androgens on the various manifestations of breast cancer are shown in Table VIII. It is clear that androgens produce a

TABLE VIII

COMPARATIVE RESPONSE TO ESTROGENS AND ANDROGENS OF VARIOUS MANIFESTATIONS OF BREAST CANCER

	*Estrogens (253)**		*Androgens (105)**	
	Per Cent	*Number*	*Per Cent*	*Number*
Subjective improvement (wellbeing)	35.0	(154)	73.8	(103)
Pain relief (osseous metastases)	42.9	(70)	81.5	(92)
Primary lesion	50.5	(95)	14.2	(14)
Soft tissue metastases	44.6	(197)	22.7	(66)
Lung metastases	40.0	(55)	7.6	(26)
Osseous metastases	22.6	(84)	23.4	(94)

* Figures in parentheses represent total number of patients with specific signs or symptoms.

much higher incidence of subjective improvement. The effects of androgens are found in patients at any stage of life. Estrogens are usually of value only in patients who are at least five years postmenopausal, whether menopause is spontaneous or artificially induced. In these cases, estrogens are obviously superior to androgens for soft tissue manifestations and essentially of equal value for osseous metastases.

Discussion

These studies and those by many others indicate that steroid hormones and allied substances may influence favorably several forms of neoplastic disease. In this respect, therefore, there appears to be an amazing specificity, indicating that a variety of etiologic factors may be concerned in any single form of disease. It appears that any agent which is therapeutically active may contribute substantially to an understanding of the etiologic aspects of a specific neoplasm. The fact that all cases of a type of hormone-susceptible cancer do not respond uniformly indicates that other factors are of great importance. It is hoped that some of

these at least might be detected by the use of other measures in addition to the steroid hormones. Finally, it appears clear that further screening of other steroids and allied compounds should be done. Care must be exercised in selecting these compounds since the number of patients is limited in any one clinic for the prosecution of such a program. It is suggested, therefore, that individuals using essentially rare compounds pool their data with others in anticipation of more rapid progress toward a common goal. One such agency is now in operation and has proved exceedingly successful in evaluating the effect of the more commonly used estrogens and androgens in breast cancer.* The scope of this study has now been expanded to include other compounds and forms of neoplastic disease.

Conclusions

The available data suggest that those steroid compounds with detectable biologic activity are most effective in causing regressions in specific types of neoplastic diseases. This does not exclude the possibility that compounds of similar structure but without obvious biologic or metabolic effects may also act favorably. Thus, the need for intensive screening of steroid and allied compounds is urgently indicated.

Discussion of Chapters 22, 23, and 24

J. Garcia Ramos: I would like to ask Dr. Hertz how many patients have undergone surgery after the progesterone treatment and what have been the pathologic reports of those studies? We have seen the results in the surface of the epithelium; this is very nice, but it is interesting to know what happened to the disease. Is the effect we have seen on the disturbed epithelium only on the surface of the epithelium, and what indeed happened deep in the tumor tissue?

R. Hertz: Fourteen of our patients were operated upon and their surgical specimens are now under study. Preliminary study of the routine pathologic material has revealed no significant change in the character of the residual tissue after regression has occurred. More detailed studies with special stains are now in progress.

F. Gomez Mont: I want to ask Dr. Nathanson how many patients responded when he increased the dose of the different steroids.

I. T. Nathanson: We have not been able to demonstrate that gross increases in the dosages of the various steroids and allied compounds usually employed will result in a significantly higher percentage of favorable responses in advanced breast cancer.

F. Homburger: The beautiful studies reported by Dr. Nathanson show clearly how difficult and time-consuming it is to evaluate the carcinolytic

* Subcommittee on Steroids and Cancer of the Committee on Research of the American Medical Association.

activities of steroids in patients with cancer of the breast. There are indeed only a very few centers where such extensive work can be done in groups of patients large enough to yield statistically significant results. It is, therefore, of the utmost importance to develop bioassay procedures which might be helpful in screening steroids for their potential carcinolytic activity against cancer of the breast. That this has so far not been possible by using transplantable tumors in mice has been shown by Dr. Stock whose bioassay screening detects only those compounds which may act upon cancer of lymphoid tissues. We feel that there is a possibility to develop other screening procedures by searching for more so-called independent actions of steroids, one or more of which might parallel carcinolytic activity of such compounds. We have proposed methylandrostenediol as a carcinolytic agent on the basis of such an independent steroid effect

Fig. 1

exhibited to a high degree by this steroid and by testosterone but not by a number of other compounds tested. This independent activity is the ability of some steroids to protect the epithelial cells in the fibrotic capsule which forms in place of the kidney after ligation of a ureter in the female mouse. In such animals there is evidence of the classic renotrophic effect upon the contralateral healthy kidney over and above the expected compensatory hypertrophy; androgenicity can be measured by the size of the clitoris or the clitoral glands, and the behavior of the renal epithelium can be studied histologically in the hydronephrotic kidney. Additional chemical studies, such as nucleic acid determinations in the damaged kidney, β-glucuronidase determinations in the contralateral kidney, and other measurements, can be done in the same animal. In C57 female mice there is a complete replacement of the renal tissue by fibroblastic proliferation 25 to 30 days after ligation of the ureter, as shown in *Fig. 1*. When testosterone propionate is given during the same period of

time in doses totaling 375 mg./kg. body weight there remain in this fibrotic tissue islands of well preserved and even proliferating renal epithelial cells *(Fig. 2)*. In *Fig. 3* several compounds so tested are arranged in order of the intensity of the classic renotrophic effect upon the contralateral healthy kidney and it is immediately apparent that in the case of testosterone propionate and methylandrostenediol the protective effect

Fig. 2

Fig. 3

upon renal epithelial cells exceeds this renotrophic action. In *Fig. 4* a number of different compounds and combinations of compounds are arranged according to increasing classic renotrophic effect and it is again seen that the protective effect upon renal epithelial cells in the hydronephrotic kidney does not parallel this renotrophic action nor does it parallel the androgenicity as measured by the weight changes of clitoris and clitoral glands. As stated before, methylandrostenediol was used by us in cancer of the breast because its behavior in this mouse test was

Fig. 4

SERIES	NUMBER PATIENTS	SUBJECTIVE IMPROVEMENT	OBJECTIVE IMPROVEMENT	NO IMPROVEMENT	HYPERCALCEMIA	VIRILISM	NAUSEA VOMITING	CONGESTION
TESTOSTERONE PROPIONATE								
CURRELL, A.R.	17	4	1	12	2	P	P	P
KEELEY, R.	23	11	5	7	2	+	4	2
TAYLOR, S.G. ET AL	19	6	5	8	2	+	P	"SEVERAL"
METHYLANDROSTENEDIOL								
KASDON, S.C. ET AL	24	12	6	6	1	0	1	NONE

Fig. 5

similar to that of testosterone propionate. Dr. Escher's report this morning confirms our initial finding that methyland stenediol is a carcinolytic agent in cancer of the breast and that it is less androgenic than testosterone propionate. In our series of 24 cases we have found *(Fig. 5)* that this compound is as effective therapeutically as testosterone propionate, with considerably less toxicity. The compound produced in our hands viriliz-

Fig. 6

Fig. 7

ing signs only when a total dose of 30 gm. was exceeded; then only hirsutism appeared but none of the other symptoms of virilization. In *Fig. 6* is shown the osteosclerosis which was found in a case of a woman with cancer of the breast who came to autopsy after having received a total dose of approximately 25 gm. of methylandrostenediol. It is evident that a marked degree of ossification occurred somewhat similar to that seen in Paget's disease of the bone. This is further illustrated in *Fig. 7,* which shows osteoblastic proliferation in wide seams of osteoid tissue. Thus the bioassay has screened out a compound which promotes bone

formation, is carcinolytic in cancer of the breast, and in metabolic studies showed marked (50 per cent of testosterone) protein anabolic effects. Since Dr. Escher's data suggest that dihydrotestosterone is also an effective carcinolytic agent in cancer of the breast it will be extremely interesting to see the results this compound will give in our bioassay. This last experiment with dihydrotestosterone may indicate whether the screening test really detects agents with possible clinical usefulness.

A. Segaloff: Because of the very obvious difficulty of giving very large amounts of progesterone, I wonder if Dr. Hertz has tried smaller amounts?

R. Hertz: All of our patients received 250 mg. daily in 5 cc. of oil. We have, however, experimented with various other routes of progesterone administration and at the moment are experimenting with vaginal suppositories, each of which contains 1 gm. of progesterone. We have in mind two factors: (1) the possibility of a high local concentration of progesterone acting directly on the cervical and pelvic tissues and (2) the possibility of increasing the dose over and above what it is possible to give intramuscularly. Our preliminary data on the pregnanediol excretion in two patients given four such suppositories daily indicate that we will get about as good return from that type of progesterone administration as we do from intramuscular and it may be possible to give large doses in that way. These studies are entirely preliminary and it is not yet time to indicate the results.

K. Dobriner: I would like to ask Dr. Hertz whether he observed a priming effect on the pregnanediol excretion after administration of progesterone? May I also ask whether Dr. Hertz has compared oil solution of progesterone with other vehicles?

We know that testosterone in the human is quickly metabolized and eliminated. Would Dr. Nathanson expect a difference between his triweekly and daily injections? The steroid investigations of Nathanson and Engel are very interesting and extremely important. One finding in the patient who received dehydroisoandrosterone appeared very significant to me. The rate of metabolism of the compound appeared to change markedly during the course of the experiment, and I wonder whether similar results have been obtained in cancer patients.

R. Hertz: We have not done much with suspensions of progesterone. The few attempts we have made indicated to us that such suspensions are highly irritating. Daily, prolonged administrations are not suitable when you get up to the level of 100 mg. per cubic centimeter. That is a problem which we do have to solve in some way.

The pregnanediol excretion studies were made on three-day pools and unlike Sommerville and Marrian who observed a steplike increment in pregnanediol excretion as progesterone administration is continued over

prolonged periods of time, we saw only very wide fluctuations from pool to pool as pregnanediol determinations were made at three-day intervals. We were not able to substantiate their observations in that regard, but I do feel that our determinations are not sufficiently quantitatively precise to say that it did not occur.

G. C. Escher: There are a few comments I would like to make with reference to the other papers. One, that I agree with Dr. Nathanson that you can't cut short the period of testing of a particular compound. We have gone as long as nine months with androgens before seeing a regression and as long as seven months with estrogens until seeing a regression. Unless you get deleterious effects, you should continue the particular compound in use.

We have also used progesterone in the treatment of breast carcinoma. Eighteen patients were treated with 100 or 200 mg. intramuscularly three times a week. Eight had no previous hormone therapy—none of these showed objective improvement. Twelve were on previous androgen therapy with a period of no hormone therapy for a variable length of time. Only three patients showed objective improvement—one for two months; another for five months, and a third for eleven months. All three had shown objective improvement on the antecedent androgen therapy.

As regards uterine bleeding during estrogen therapy, we have found that if a patient develops such bleeding on a particular estrogen compound, increasing the dosage of the estrogen will stop the bleeding. We also agree with Dr. Nathanson as to a discrepancy in response of the same areas of metastases to androgens as opposed to estrogens; we have almost the same percentages. We have already started a series of patients on daily rather than thrice weekly injections of various hormones with the hope that we can show a higher percentage of objective improvement.

I would like to bring up one point that no one has mentioned at all in the course of this entire program and that is the hematopoietic response to hormones and the divergence of effect between estrogens and androgens. Androgens will produce a perceptible increase in the red blood cell factors without necessarily any correlation to objective improvement in the cancer. The increase can reach polycythemic levels. Others just show an improvement in red blood cell count, hemoglobin, and hematocrit. Estrogens do the reverse even in the presence of objective improvement in a certain number of cases; in other words, a depression of these particular aforementioned blood factors.

E. B. Astwood: I would like to ask Dr. Hertz two questions. In the first place, it used to be said that carcinoma of the cervix extends rapidly during pregnancy. Would Dr. Hertz tell us whether this is correct, and, if so, whether it means that progesterone is not the agent responsible for it. In the second place, I would like to ask Dr. Hertz whether he plans to carry out control experiments wherein patients with carcinoma of the

cervix are subjected to the same manipulative procedures but without any treatment.

R. Hertz: My primary consideration in presenting the data on the histidinuria was to indicate that the administration of massive doses of progesterone do not reproduce the endocrine status of the pregnant individual. Now, with respect to the exacerbation of cervical carcinoma during pregnancy there is mixed opinion in the literature. I have no direct clinical experience with the problem but in talking with many gynecologists I can get no concurrence of opinion and I believe there has been no objective detailed study of the effect of pregnancy on cervical lesions.

As a matter of clinical impression, some gynecologists seem to think that pregnancy is unfavorable, and some feel that it is all right to watch the lesion through pregnancy if the child is wanted badly enough. There appears to be considerable difference of opinion.

With respect to a control period with similar manipulations of the cervix, I might say that prior to the institution of progesterone studies, very similar observations were made, in not too systematic a way, while patients were awaiting surgery. Under practically identical conditions, then, with those that we have maintained for our progesterone-treated patients, we saw no such regressive changes.

I. J. Nathanson: Although the number of cases is small, our data indicate that patients who develop cancer of the cervix during pregnancy have a much poorer prognosis than comparable nonpregnant women who are subjected to the same treatment. It is our impression that during lactation the disease grows more rapidly or exacerbates after an apparent arrest if treated during pregnancy.

R. H. Freyberg: Since there is different sexogenic effect from estrogens and androgens and differences in their effect on the different types of neoplastic disease, are there different effects, especially in their therapeutic value, in alternating androgen and estrogen treatment or in their simultaneous use?

G. C. Escher: I think I speak for most of the group who have experience with steroid hormone therapy in breast carcinoma. We have found that when a regression occurs with this type of tumor on hormone therapy, continuation of hormone therapy results in eventual progression. When we stop therapy we may get another period of regression and then if, after that period of no therapy, we start the antagonist type of compound, we may get another period of regression. We have tried a few cases on combined estrogen and androgen and have seen no particular advantage. I don't know if Dr. Nathanson has tried any more or has seen any better results.

I. T. Nathanson: The observations of Drs. Adair and Escher are in agreement with those made by our group as regards another remission following cessation of therapy and again after administration of the opposite type of hormone. Estrogens and androgens have been used simultaneously, alternately, and successively but we have been unable to establish an advantage for any one routine over the predicted effect of a single compound. We had hoped that we might observe a synergistic effect especially as regards osseous metastases. Perhaps additional observations in this direction may clarify the issue.

At this point some observations relative to combined use of androgens and cortical steroids may be pertinent to future studies. This is a study of estrogen and 17-ketosteroid excretion rates in a male arthritic patient treated with testosterone, adrenocortical steroids, and combinations of these compounds. The data in the following table show the effects

TABLE I

THE EFFECT OF TESTOSTERONE AND ADRENAL CORTICAL STEROIDS ON THE EXCRETION OF KETOSTEROIDS AND ESTROGENS IN A PATIENT WITH RHEUMATOID ARTHRITIS

Compound (mg./day)	*% Change from Control Levels*	
	Ketosteroids	*Estrogens*
Desoxycorticosterone acetate (25)	− 6	0
Cortisone acetate (150)	− 49	− 12
Testosterone (100)	+174	+ 26
Desoxycorticosterone acetate (25), cortisone acetate (150)	− 36	+ 9
Testosterone propionate (100), desoxycorticosterone acetate (25)	+216	+ 15
Testosterone (100), cortisone acetate (150)	+296	+100
Testosterone propionate (100), cortisone acetate (150), desoxycorticosterone acetate (25)	+167	+ 24

of testosterone and two adrenal cortical compounds on the urinary excretion of steroids in one of Dr. Bauer's patients with rheumatoid arthritis. Cortisone alone or in combination with desoxycorticosterone diminishes the urinary output of ketosteroids and has no significant effect on estrogen excretion. Testosterone alone causes an increased excretion of both ketosteroids and estrogens. When cortisone is administered with testosterone, there is a marked enhancement of these effects. Combination of desoxycorticosterone with testosterone leads to an excretion of ketosteroids somewhat greater than with testosterone alone and a less marked rise in estrogen excretion than with testosterone alone. When all three steroids are given together the net effect on both ketosteroid and estrogen excretion is similar to that of testosterone alone. Insofar as the effect on

the excretion of estrogens is concerned, cortisone seems to enhance and desoxycorticosterone seems to depress the formation and excretion of these compounds following testosterone administration. Both desoxycorticosterone and cortisone seem to enhance the production of ketosteroids from testosterone, but the combination of the three hormones leads to an excretion of ketosteroids similar to that found when testosterone alone is administered.

A. Segaloff: There is one thing I would like to bring out which Dr. Escher just stopped short of mentioning. That is the finding that other compounds of low activities against mammary cancer give good responses in the patients who have responded either previously or afterwards to testosterone. This would appear to me to emphasize the fact that there is something about the patient and possibly something about the tumor that makes them more susceptible to a wide variety of steroidal compounds; perhaps not just the previous testosterone therapy.

I. T. Nathanson: I would like to end this discussion with a few comments particularly concerning the remarks of Dr. Segaloff regarding the type of patient and tumor that might be anticipated to be more receptive to steroid therapy. Generally, the slowly growing breast or prostatic cancer responds best to steroids even though the disease may be extensive at the onset of therapy. By contrast, we are all familiar with occasional small and apparently early cancers of the breast and prostate gland which theoretically should be controlled by radical surgery, but which recur rapidly and metastasize without bounds after such treatment. These lesions are seldom amenable to steroid therapy. These facts imply that an inherent resistance in the host and the biologic character of the tumor are important factors in the course of a particular tumor. Thus, modification of the course of a tumor by steroids certainly seems to depend to a considerable extent upon these factors. The histologic appearance of the tumor does not supply the information needed to predict the responsiveness of a tumor to steroids. It is clear, therefore, that more precise techniques at the local and systemic levels are necessary to gain an insight into the fundamental factors concerned in the growth and regressive phases of cancer.

Summary Table I

Clinical Studies with Pregnenolone and Pregnenolone Acetate

Clinical Condition (showing state of disease where given)	*Route of Administ.* †	*Dosage Form* ‡	*Dosage Schedule* *Mg./Day*	*Duration Days*	*Avg. Total Dose-Gm.*
Rheumatoid arthritis	O	T	400– 600	34	19.0
Mild—Stage I	O	T	375	28	10.5
	O	T	500; 800	46; 31	23.0; 27.3
Totals	O, I.M.	T, A.S.	400–1500	8–169	52.4
Moderate—Stage II	O, I.M.	T, A.S., O.S.	100– 900	20–154	38.0
	O	T	350– 400	35–104	23.8
	O	T	500	49; 55	24.5; 27.5
	O, I.M.	T, A.S.	100– 600	29– 30	20.9
	O	T	500	14– 30	6.0–15.0
Totals					
Severe—Stages III & IV	O, I.M.	T, O.S., A.S.	100– 900	15–276	28.5
	O	T	300– 400	17– 74	14.1
	O	T	425– 850	21– 71	18.8
	O, I.M.	T, A.S.	200–1000	12–123	24.3
	O	T	500	14– 30	6.0–15.0
Totals					
Unclassified§	O, I.M.	T, O.S., A.S.	100– 600	540	—
	O, I.M.	T, A.S.	235–1000	4– 11	5.8
	I.M.	A.S.	300– 400	16– 30	15.0
	O, I.M.	T, O.S., A.S.	100– 400	14–120	15.5
	O	T, A.S.	800	21– 63	33.6
Totals					
Spondylarthritis	O, I.M.	T, A.S., O.S.	100– 600	10– 41	33.5
	O	T	400; 500	33; 40	13.7; 20.0
	O	T, A.S.	800	27– 63	32.8
Totals					

*Major improvement includes clinical responses classified in grade I according to A.R.A. standards, and evaluated by the authors as showing either complete remission or objective improvement. Minor improvement includes all patients classified as grades II and III.

†O—oral route I.M.—intramuscular injection

‡T—tablets for oral ingestion A.S.—aqueous suspension O.S.—oil solution

SUMMARY TABLE I—*Continued*

CLINICAL STUDIES WITH PREGNENOLONE AND PREGNENOLONE ACETATE

*No. of Patients Treated and Evaluation of Clinical Response**					
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*
1			1		12 Davison
1			1	Subjective improvement only.	15 Freyberg et al.
2		1	1	Patient on larger dose showed subjective improvement and class III (A.R.A.) response.	15 Freyberg et al.
4	3		1		18 McGavack et al.
8	3	1	4		
13	5	4	4	See author's tables for detailed analysis of clinical findings.	12 Davison
5		1	4		15 Freyberg et al.
2		1	1		15 Freyberg et al.
15	8		7		18 McGavack et al.
32	11	18	3		13 Freeman et al.
67	24	24	19		
38	16	10	12	See author's tables for detailed analysis of clinical findings.	12 Davison
11		2	9	One of two cases of minor improvement treated 74 days, 400 mg./day.	15 Freyberg et al.
15		8	7	In one case improvement moderate (grade II, A.RA.).	15 Freyberg et al.
21	9		12		18 McGavack et al.
67	23	23	21		18 Freeman et al.
152	48	43	61		
142	37		105	Clinical responses not graded; figures relate to no. of patients showing continued remission for 1 yr. Some patients received testosterone as adjuvant therapy.	11 Hellbaum et al.
3			3		20 Danowski et al.
10		2	8	Painful injection, 2; edema, 1.	15 Freyberg et al.
31	18	5	8	Of 18 listed under major improvement, only 7 classified as objective improvement.	19 Robles Gil et al.
8	1		7	5 of 7 listed as no response showed subjective improvement.	17 Holbrook et al.
194	56	7	131		
8	4	2	2		12 Davison
2			2		15 Freyberg et al.
2	2				17 Holbrook et al.
12	6	2	4		

§The cases placed in the unclassified category of rheumatoid arthritis include those which were not graded as to severity of disease at beginning of therapy, and those which, although classified in some instances initially, were grouped or discussed together with respect to therapeutic response.

SUMMARY TABLE I—*Continued*

CLINICAL STUDIES WITH PREGNENOLONE AND PREGNENOLONE ACETATE

Clinical Condition (showing state of disease where given)	*Route of Administ.*†	*Dosage Form*‡	*Dosage Schedule*		
			Mg./Day	*Duration Days*	*Avg. Total Dose-Gm.*
Acute rheumatic fever with carditis	O, I.M.	T, A.S.	500–1000	4– 21	7.5
Osteoarthritis:					
Stage I	O, I.M.	T, A.S.	400– 600	8– 89	20.2
Stage II	O	T	60– 400	16–120	8.5
Stage III	O	T	100– 400	17–132	19.6
Stage IV	O	T	200– 600	8– 60	19.2
Totals					
Disseminated lupus erythematosis	O	T	235–1000	8– 21	8.9
	O, I.M.	T, A.S.	200– 600	2– 81	6.3
Totals					
Fibrositis	O	T	600–1000	21– 84	42.0
Subdeltoid bursitis	O, I.M.	T	400– 800	6– 14	6.0
Seminal inadequacy	I.M.	O.S.	50– 100	28– 56	3.0
	O	T	50– 100		
	I.M.	A.S.			
Gout	O, I.M.	T, A.S.	90– 300	8– 60	4.0
Allergic hydrarthrosis	O, I.M.	T, A.S.	400– 600	25	14.6
Acute arthritis	O, I.M.	T, A.S.	50– 200	27	2.3
Psoriasis	O, I.M.	T, A.S.	200– 600	20–197	15.4
Scleroderma	O, I.M.	T, A.S.	200– 400	15–150	14.1
Chorioretinitis	O, I.M.	T, A.S.	400	74	29.6
Malignant exophthalmos	O, I.M.	T, A.S.	100– 600	21–150	17.2
Addison's disease	O, I.M.	T, A.S.	100– 300	4–167	4.7
Hyperthyroidism	O, I.M.	T, A.S.	100	30	3.0
Myxedema	O, I.M.	T, A.S.	200– 400	23	6.8
Chronic cystic mastitis	O, I.M.	T, A.S.	50– 300	180	21.1
Obesity	O, I.M.	T, A.S.	100– 400	90	11.1
Hodgkin's disease	O, I.M.	T, A.S.	300	20	6.0
Chronic pancreatitis	O, I.M.	T, A.S.	150– 300	21– 92	19.2
Tuberculosis, spine	O, I.M.	T, A.S.	100– 400	30	5.5
Amyotrophic lateral sclerosis	O, I.M.	T, A.S.	400	240	56.0
Epilepsy	O, I.M.	T, A.S.	300	8	2.4
Erythema nodosum	O, I.M.	T, A.S.	400	19	7.6

*Major improvement includes clinical responses classified in grade I according to A.R.A. standards, and evaluated by the authors as showing either complete remission or objective improvement. Minor improvement includes all patients classified as grades II and III.

†O—oral route I.M.—intramuscular injection

‡T—tablets for oral ingestion A.S.—aqueous suspension O.S.—oil solution

SUMMARY TABLE I—*Concluded*

CLINICAL STUDIES WITH PREGNENOLONE AND PREGNENOLONE ACETATE

*No. of Patients Treated and Evaluation of Clinical Response**						
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*	
3	1	1	1		20	Danowski et al.
2	1		1	The total of 16 cases listed as major improvement are classified by authors as "Marked to moderate improvement."	18	McGavack et al.
12	7		5		18	McGavack et al.
14	8		6		18	McGavack et al.
3			3		18	McGavack et al.
31	16		15			
2	1	1		Trend toward some degree of spontaneous remission had been evident prior to therapy in patient who showed complete remission with pregnenolone.	20	Danowski et al.
9	4		5	3 acute, 6 subacute or chronic. Good response in subacute or chronic cases.	18	McGavack et al.
11	5	1	5			
50	29	16	5		14	Smith
10	7	1	2	See author for details of regimen.	14	Smith
40	24	13	3	Data on conceptions and other aspects given by author.	21	Abarbanel
3	1	1	1		21	Abarbanel
4			4		21	Abarbanel
3	1		2		18	McGavack et al.
1			1		18	McGavack et al.
1	1				18	McGavack et al.
4	2		2		18	McGavack et al.
3	2		1		18	McGavack et al.
1	1				18	McGavack et al.
5	5				18	McGavack et al.
6			6		18	McGavack et al.
1			1		18	McGavack et al.
1			1		18	McGavack et al.
1			1		18	McGavack et al.
1			1		18	McGavack et al.
1			1		18	McGavack et al.
2			2		18	McGavack et al.
1			1		18	McGavack et al.
1	1				18	McGavack et al.
1			1		18	McGavack et al.
1			1		18	McGavack et al.

§The cases placed in the unclassified category of rheumatoid arthritis include those which were not graded as to severity of disease at beginning of therapy, and those which, although classified in some instances initially, were grouped or discussed together with respect to therapeutic response.

Summary Table II

Clinical Studies with Pregnenolone and Pregnenolone Acetate as Adjuvants to or Following Cortisone or ACTH

Clinical Condition (showing state of disease where given).	*Route of Administ.†*	*Dosage Form‡*	*Dosage Schedule*		
			Mg./Day	*Duration Days*	*Avg. Total Dose-Gm.*
Rheumatoid arthritis Stages I to IV	I.M.	O.S.	300	10	3.0
	I.M.	O.S.	300	10	3.0
	I.M.	O.S.	300	15– 20	5.2
Stages II & III	O	T	600	14– 21	10.5
Unclassified§	O	T	800	14– 63	30.8

*Major improvement includes clinical responses classified in grade I according to A.R.A. standards, and evaluated by the authors as showing either complete remission or objective improvement. Minor improvement includes all patients classified as grades II and III.

†O.—oral route I.M.—intramuscular injection

‡T—tablets for oral ingestion A.S.—aqueous suspension O.S.—oil solution

SUMMARY TABLE II—*Concluded*

CLINICAL STUDIES WITH PREGNENOLONE AND PREGNENOLONE ACETATE AS ADJUVANTS TO OR FOLLOWING CORTISONE OR ACTH

*No. of Patients Treated and Evaluation of Clinical Response**					
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*
4			4	Patients received either 100 mg. of cortisone orally or pregnenolone on alternate days. Improved on cortisone; relapsed on pregnenolone.	19 Robles Gil et al.
3			3	Patients received either 80 mg. of ACTH by injection or pregnenolone on alternate days. Improved on ACTH; relapsed on pregnenolone.	19 Robles Gil et al.
3	See Comments			2 patients received cortisone 100 mg. orally, one 80 mg. of ACTH by injection simultaneously with pregnenolone. Patients required less cortisone on combined therapy; there was no effect on arthritis with ACTH and pregnenolone.	19 Robles Gil et al.
24	See Comments			9 patients received ACTH 15-60 mg./day for 9 days followed by pregnenolone. 7 relapsed on pregnenolone or when ACTH dose was reduced. 9 patients received ACTH 60 mg./day for 6 days followed by pregnenolone or placebo. 5 had major response on pregnenolone, 4 on placebo. 6 patients received 100-300 mg. cortisone daily for 1-4 days followed by pregnenolone for 21 days. 3 had major response with pregnenolone, 1 with placebo. 1 had minor response with placebo. 2 had no response with cortisone with placebo.	16 Stecher et al.
17	8		9	Cortisone given orally in suboptimal amounts 25-50 mg. daily with pregnenolone. 12 patients had subjective improvement, in addition to objective improvement in the 8 listed.	17 Holbrook et al.

§The cases placed in the unclassified category of rheumatoid arthritis include those which were not graded as to severity of disease at beginning of therapy, and those which, although classified in some instances initially, were grouped or discussed together with respect to therapeutic response.

Summary Table III

Clinical Studies with 21-Acetoxypregnenolone

Clinical Condition (showing state of disease where given)	Route of Administ.†	Dosage Form‡	Dosage Schedule Mg./Day	Duration Days	Avg. Total Dose-Gm.
Rheumatoid arthritis					
Stage II	I.M.	A.S.	100– 400	14– 42	7.0
Unclassified§	I.M.	A.S.	1000	5– 7	6.0
Chronic rheumatic heart disease with congestive heart failure	I.M.	A.S.	1000	10	10.0
Acute leukemia					
Lymphatic	I.M.	A.S.	1000	31	31.0
Myelogenous	I.M.	A.S.	1000	8	8.0

Summary Table IV

Clinical Studies with Androgens, Estrogens, and Progesterone

Clinical Condition (showing state of disease where given)	Route of Administ.†	Dosage Form‡	Dosage Schedule Mg./Day	Duration Days	Avg. Total Dose-Gm.
Rheumatoid arthritis					
Stages II & III					
11-Hydroxyprogesterone	I.M.		225– 300	11– 12	2.35; 2.75
Testosterone	I.M.		not detailed	not detailed	
Estradiol	I.M.		not detailed	not detailed	
Unclassified§					
Testosterone			10– 45	540	14.8
Testolactone	O		400	14– 49	8.4
Dihydroisoandrosterone acetate	O		200– 500	14– 49	10.9
Marisone (Ayerst)	O		1000–2000	7– 56	46.5
Δ^5, 17 Ethinylandrostene, 3, 17-diol	I.M.		100– 200	4– 8	1.0
Seminal inadequacy					
Testosterone propionate	I.M.	O.S.	5-25/wk.	28– 84	0.11
Methyl testosterone	O	T	35-70/wk.	28– 56	0.30
Ethinyl testosterone	O	T	5– 30	28– 56	0.7
Progesterone	O, I.M.	T, O.S.	5-10/wk.	28– 84	0.05
Carcinoma of cervix					
Progesterone	I.M.	O.S.	250	10–170	22.0
Mammary carcinoma					
Dihydrotestosterone	I.M.		100		
Dihydrotestosterone acetate	I.M.		100		
Methylandrostenediol	I.M.		100		

*Major improvement includes clinical responses classified in grade I according to A.R.A. standards, and evaluated by the authors as showing either complete remission or objective improvement. Minor improvement includes all patients classified as grades II and III.
†O—oral route I.M.—intramuscular injection

SUMMARY TABLE III—*Concluded*

CLINICAL STUDIES WITH 21-ACETOXYPREGNENOLONE

*No. of Patients Treated and Evaluation of Clinical Response**						
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*	
9		3	6		15	Freyberg et al.
3				Clinical response not evaluated; therapy discontinued because of local reactions.	20	Danowski et al.
1		1			20	Danowski et al.
1		1			20	Danowski et al.
1			1		20	Danowski et al.

SUMMARY TABLE IV—*Concluded*

CLINICAL STUDIES WITH ANDROGENS, ESTROGENS, AND PROGESTERONE

*No. of Patients Treated and Evaluation of Clinical Response**						
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*	
2			2		15	Freyberg et al.
12			12		15	Freyberg et al.
6			6		15	Freyberg et al.
124	53		71	Testosterone propionate given either alone or together with pregnenolone.	11	Hellbaum et al.
11			11		13	Freeman et al.
8			8		13	Freeman et al.
30		11	19	Improvement slight in 11 cases; nausea; evidence of estrogenic activity.	13	Freeman et al.
3			3		15	Freyberg et al.
8	1	1	6		21	Abarbanel
10	8		2		21	Abarbanel
5			5		21	Abarbanel
8			8		21	Abarbanel
17	11	6			22	Hertz et al.
6	6				23	Escher et al.
2	2				23	Escher et al.
5	2		2	1 patient taken off therapy after 1 week.	23	Escher et al.

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Summary Table V

Clinical Studies with Miscellaneous Steroids

Clinical Condition (showing state of disease where given)	*Route of Administ.* †	*Dosage Form* ‡	*Dosage Schedule*		
			Mg./Day	*Duration Days*	*Avg. Total Dose-Gm.*
Rheumatoid arthritis					
Δ^4-Pregnene, 17α, 20β, 21-triol, 3-one (Pregnene Triolone)	I.M.		135– 250	4– 14	1.5
	O		300– 500	7– 28	6.8
Totals					
Δ^4-Pregnene, 17α, 20β, 21-triol, 3-one, 20, 21-diacetate	I.M.		175– 200	6– 11	1.6
Δ^{17}-Pregnene, 3, 21, diol-11-one, 20-cyano	I.M.		70; 125	3; 17	0.29; 2.0
Dihydrocortisone	I.M.		100– 200	2– 12	0.7
Δ^4-Pregnene, 17α, 21-diol-3, 20-dione 21 acetate (Reichstein's S acetate)	I.M.		300	8– 20	4.2
Δ^4-Androstene-dione, 3, 17	I.M.		200	6– 12	1.0–1.7
	O		500–1000	7– 28	3.5–28.0
Totals					
Δ^5-3-Acetoxyetiocholenic acid	I.M.		100– 200	16	2.0
Δ5, 16-Pregnadiene 3β-ol-20-one acetate	O		600	21– 35	20.0
	O		500–1000	7– 42	9.5
Totals					
Allopregnane 3β 21-diol-20-one diacetate	O		400– 600	21– 84	26.0
Allopregnane-21, ol-3, 20-dione acetate	O		400	21– 42	12.5
	I.M.		145– 215	7– 12	1.7
Totals					

*Major improvement includes clinical responses classified in grade I according to A.R.A. standards, and evaluated by the authors as showing either complete remission or objective improvement. Minor improvement includes all patients classified as grades II and III.
†O—oral route I.M.—intramuscular injection
‡T—tablets for oral ingestion A.S.—aqueous suspension O.S.—oil solution

SUMMARY TABLE V—*Concluded*

CLINICAL STUDIES WITH MISCELLANEOUS STEROIDS

*No. of Patients Treated and Evaluation of Clinical Response**						
No. Treated	*Major Improvement*	*Minor Improvement*	*No Improvement*	*Comments*	*Chapter and Author*	
4			4		15	Freyberg et al.
9			9		13	Freeman et al.
13			13			
3			3		15	Freyberg et al.
2			2		15	Freyberg et al.
5			5	Various undesirable side-effects.	15	Freyberg et al.
3			3		15	Freyberg et al.
3			3		15	Freyberg et al.
33			33		13	Freeman et al.
36			36			
1	1				15	Freyberg et al.
11	2	3	6			
19	1	5	13		17	Holbrook et al.
				Only 3 patients received 1000 mg. dose.	13	Freeman et al.
30	3	8	19			
13	4	6	3	2 patients showing major improvement had drop in sedimentation rate.	17	Holbrook et al.
11	2	3	6		17	Holbrook et al.
6		2	4		15	Freyberg et al.
17	2	5	10			

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