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Perspectives in Biology and Medicine, Volume 13, Number 3, Spring 1970, pp. 343-363 (Article)

Published by Johns Hopkins University Press
DOI: *https://doi.org/10.1353/pbm.1970.0055*

ADAPTIVE STEROIDS: RETROSPECT AND PROSPECT

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The important role of steroid hormones in the regulation of growth, reproduction, and general metabolism has long been recognized; yet it is only during the last thirty years or so that we became aware of their participation in diverse nonspecific adaptive reactions, and particularly in the maintenance of the body's resistance to stress. This review attempts to give (1) a brief introductory outline of what we believe to be a natural basis for the classification of adaptive steroids, (2) a survey of the historic development of this topic with special reference to the recently characterized "catatoxic" steroids, and (3) an outlook assessing the future possibilities of research along these lines.

Classification

It has been found convenient to classify these adaptive hormones and their derivatives into two main groups which control essentially different defensive processes [1]:

A. *The syntoxic steroids* (e.g., cortisol, triamcinolone, aldosterone, desoxycorticosterone) initiate changes which permit life under stress without directly attacking the damaging agent. They create conditions for coexistence with the aggressor either through passive indolence to it (e.g., antiphlogistics) or by actively stimulating the formation of a

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This work was supported by the Ministère de la Santé, Québec, and the Medical Research Council of Canada (Block Term grant MT-1829). I wish to thank the following companies for kindly supplying the compounds used in these experiments: E. R. Squibb & Sons Ltd., G. D. Searle & Co., Lederle Laboratories, Merck & Co. Inc., Organon Inc., Schering Corporation Inc., Schieffelin & Co., Smith, Kline & French Laboratories, Sterling-Winthrop Research Institute, Syntex Research, and the Upjohn Co.

granulomatous barricade, which tends to isolate the irritant from the surrounding tissue (e.g., prophlogistics). Through similar mechanisms, the syntoxic steroids also promote repair (e.g., cicatrization, anabolism) or help to eliminate the pathogen (e.g., evacuation through the formation of a perforating abscess), but they do not destroy it.

The systemic action of the syntoxic steroids is mainly of the "life-maintaining corticoid" type; it is highly efficient in restoring the non-specific stress resistance of adrenal-deficient organisms to normal, but then a plateau is reached above which tolerance cannot be raised easily. Only in some instances (e.g., damage due to endotoxins, inflammatory irritants, immune reactions, lathyrogenic compounds) do syntoxic steroids increase tolerance far above normal, because here "disease" is primarily due to active morbid reactions of the tissues, not to direct tissue damage by the exogenous aggressor. Thus, endotoxin shock is thought to be caused mainly by the liberation of enzymes normally sequestered in lysosomes, whereas inflammation, various pathogenic immune reactions (allergies, anaphylaxis, homograft rejection), and osteolathyrism represent excessive responses of the body to different types of irritation.

Finally, it must be remembered that some protective actions are based on specific pharmacologic antagonisms between steroids and toxic substances (e.g., mineralocorticoid-induced potassium tolerance, glucocorticoid-induced insulin tolerance). Here again, homeostasis is achieved by adjusting the body's reaction to the damaging agent, not by destroying the latter.

B. *The catatoxic steroids* (e.g., ethylestrenol, spironolactone) act primarily by inducing aggressive reactions which inactivate toxic substances (e.g., by accelerating their metabolic degradation). They not only restore a deficient resistance to normal (as do the glucocorticoids after adrenalectomy), but are capable of raising it far above the norm. Sometimes this reaction defeats its purpose, because the products of metabolic degradation are more toxic than the original poison which was to be inactivated. Yet the response is still catatoxic since it attacks the toxic aggressor. For similar reasons we speak of allergy and anaphylaxis as "immune reactions," although they actually produce damage.

There are many overlaps between syntoxic and catatoxic steroid actions. Thus, stimulation of inflammation may lead to topical degradation of the

irritant by enzyme activation in the inflammatory focus; furthermore, under certain circumstances the primarily syntoxic glucocorticoids may enhance the hepatic detoxification of barbiturates. Yet, the distinction between these two categories appears to be as justified as, say, the distinction between glucocorticoids and mineralocorticoids, because as a rule individual steroids act predominantly by eliciting one or the other reaction form.

Furthermore, available evidence suggests that the two types of defense are mediated through essentially distinct mechanisms. For example, as stated above, homeostatic reactions to endotoxins or inflammatory irritants appear to depend upon the stabilization of membranes which isolate toxic enzymes that are preformed within the cell. In so doing they protect against damage resulting from a kind of autointoxication by natural substances liberated under the influence of an aggressor. Conversely, many catatoxic hormones have been shown to induce NADPH-dependent hepatic microsomal enzymes which destroy endogenous or exogenous toxic substances. Yet, without further evidence, it would be hazardous to equate this mechanism with the catatoxic action. It is already evident that not all catatoxic steroids inactivate the same set of substrates, and it will be necessary in each case to determine that the detoxification occurs (1) in the liver, (2) in the microsomal fraction, (3) as a consequence of NADPH-dependent enzymes, and (4) that these enzymes have been induced *de novo* and not merely activated. Hence, it is more prudent meanwhile to recognize the possibility of other catatoxic mechanisms and to ascribe detoxification reactions to the induction of NADPH-dependent hepatic microsomal enzymes only whenever this has been definitely proven. Figure 1 will help to illustrate the classification used in this review.

These considerations have been useful in delimiting the catatoxic effect from other independent steroid hormone actions (e.g., corticoid, testoid, folliculoid, luteoid, anesthetic), but before applying the concept certain sources of misunderstanding must be discussed.

Although the catatoxic action is independent of other pharmacologic properties, individual catatoxic compounds may, and usually do, exhibit additional effects. Most of the potent catatoxic steroids are also endowed with antimineralocorticoid, anabolic, or glucocorticoid properties, but their effect upon resistance is not inseparably linked to any one of these associated properties. Thus, ethylestrenol and spironolactone are among

the most active catatoxic steroids, yet the former is almost exclusively anabolic, the latter antimineralocorticoid, and neither of them possesses glucocorticoid potency.

The nonspecificity of the catatoxic effect is not absolute. Steroids endowed with this property induce tolerance to many agents, widely different in their chemical structure and pharmacologic activity; yet, there are numerous injurious substances whose effect is not diminished by pretreatment with catatoxic steroids. In this respect, the protective effect of the latter is much less nonspecific than that of the "life-maintaining corticoids." The "activity spectrum" of the catatoxic steroids varies; some

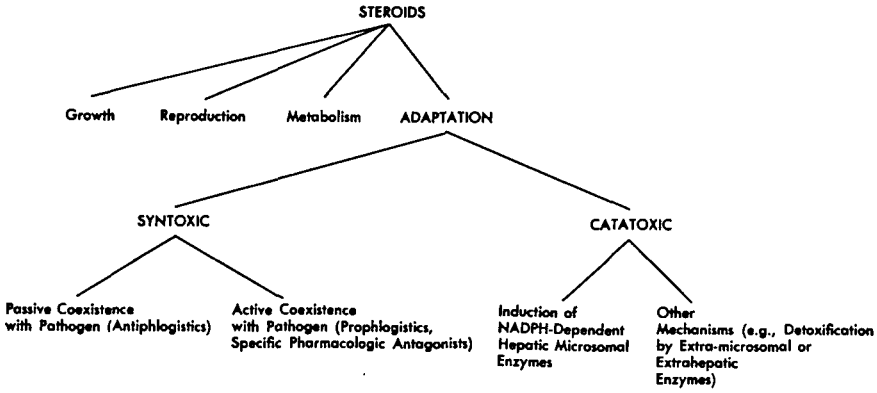

FIG. 1.—Classification of the pharmacologically active steroids

(e.g., ethylestrenol, SC-11927) are efficacious against numerous toxic substances, whereas others (e.g., prednisolone, progesterone) offer protection only against few agents.

This concept has developed gradually over a period of almost four decades, and it may be useful now to trace its evolution in retrospect and to appraise its possible future prospects.

History

It must be made clear from the outset that many seemingly unrelated experiments have led to the formulation of the idea that certain steroids are especially concerned with defense against toxic agents. The story of the syntoxic (especially the antiphlogistic) steroids is sufficiently known, so that we may limit ourselves here to a discussion of investigations on the

hormonal regulation of detoxification. This work was not guided by a preconceived master plan. It is only with the hindsight of today's knowledge that we can identify the steps that gradually lead to the emergence of the catatoxic steroid concept as we see it today.

The main stages in this process were based on the following observations:

1. Partial hepatectomy in small laboratory rodents helps to identify drugs that are detoxified by the liver.
2. The body can develop resistance to its own steroid hormones.
3. Sex hormones influence resistance to many drugs.
4. Induced hepatic detoxification of drugs can be demonstrated in vitro by incubation of the substrate with liver tissue.
5. Induced hepatic detoxification of drugs in vitro depends largely upon the production of appropriate microsomal enzymes.
6. Proliferation of the smooth-surfaced endoplasmic reticulum (SER) in hepatocytes may reflect enzyme induction.
7. Numerous apparently quite unrelated pathologic changes are preventable by various types of steroids in intact animals.
8. Recognition of "catatoxic steroids" as an independent pharmacologic group.

1. PARTIAL HEPATECTOMY IN SMALL LABORATORY RODENTS AS A MEANS OF IDENTIFYING DRUGS THAT ARE DETOXIFIED BY THE LIVER

As the "central chemical laboratory of the body," the liver has long been suspected of playing an important part in the inactivation of exogenous and endogenous toxic substances. However, large-scale systematic studies, designed to identify the compounds subject to hepatic detoxification, were virtually impossible because of the lack of appropriate techniques. Comparisons between the drug resistance of intact and hepatectomized animals were difficult to interpret, since complete removal of the liver causes severe shock rapidly terminating in death, especially when toxic substances are given, irrespective of whether or not these are amenable to hepatic detoxification. Animals in which partial hepatic insufficiency was created (e.g., by ligature of the bile duct, hepatotoxic drugs, or the establishment of an Eck fistula) likewise yielded variable results, often complicated by damage to extrahepatic tissues. Finally, the search for presumed drug metabolites in hepatic vein-blood in vivo did not lend

itself to the screening of many drugs, whereas similar studies on liver perfusates in vitro often failed to reflect in vivo conditions.

In order to test hepatic participation in the detoxification of numerous compounds, a screening test became necessary. It was to answer this need that we devised a simple surgical technique for the ablation of the left lateral and median lobes of the liver in mice [2]. This operation removes about 70 percent of the hepatic tissue and markedly reduces resistance only with respect to drugs detoxified by the liver. For such tests it is best to use the animals about twenty-four hours after the intervention, when they have recovered from the surgical insult but hepatic regeneration is still negligible. With this technique we showed, for example, that the partially hepatectomized mouse is extremely sensitive to the anesthetic effect of tribromoethanol (Avertin), which is detoxified by the liver, but not to that of an equally anesthetic dose of $MgCl_2$, which is not subject to hepatic detoxification. Almost at the same time, Higgins and Anderson [3] recommended an essentially similar operation for the stimulation of hepatic regeneration in the rat. They did not attempt to use partial hepatectomy for detoxification studies, but noted that regeneration is virtually complete after two weeks.

About ten years later, the site of steroid hormone detoxification had become a major subject of controversy, difficult to solve with the chemical methods then available. However, in the meantime we had observed that sudden overdosage with steroid hormones and their derivatives produces profound anesthesia in the rat [4]. This indication of activity was clear-cut, common to virtually all steroid hormones, not particularly damaging, and almost immediately evident; thus it was applicable to acute experiments on partially hepatectomized rats before regeneration could become important. Hence, we injected threshold doses of desoxycorticosterone, progesterone, testosterone, and estradiol into intact and partially hepatectomized rats. The latter proved to be unusually sensitive to all these steroids, whereas their resistance to several other anesthetics remained uninfluenced. Even overdosage with the nonsteroidal folliculoid, stilbestrol (which normally causes only a very mild hypnotic effect), produced prolonged narcosis after partial hepatectomy. These observations lead us to conclude that "it appears most probable that the liver is the site at which all the above-mentioned compounds are normally detoxified" [5].

Subsequent investigations have amply confirmed the importance of

the liver as the organ principally responsible for the detoxification of steroid hormones, as well as the value of partial hepatectomy as a simple screening test for compounds whose actions largely depend upon the speed of their hepatic detoxification. Furthermore, recently we have seen (in agreement with expectations) that following partial resection of the liver, drugs against which catatoxic steroids can offer protection through hepatic microsomal enzyme induction become particularly toxic. However, the steroidal enzyme inducers themselves are also subject to hepatic detoxification, and consequently their catatoxic activity likewise increases when their metabolic degradation is impeded by partial hepatectomy. Thus, in the rat, this operation facilitates both the production of perforating intestinal ulcers by indomethacin (a substrate for hepatic detoxification) and the prevention of these lesions by small doses of a catatoxic steroid such as spironolactone [6].

In evaluating the results of partial hepatectomy upon drug toxicity it must be kept in mind, however, that the liver may also participate in the defense against toxic substances through mechanisms unrelated to the induction of microsomal enzymes (e.g., synthesis of energy yielding metabolites, elimination of pathogens through the bile, or their storage in the RES). Hence, in any one case, aggravation of drug toxicity by partial hepatectomy merely suggests, but does not prove, that resistance may be increased by catatoxic steroid.

2. THE BODY CAN DEVELOP RESISTANCE TO NATURAL STEROID HORMONES

It is well established that proteinaceous extracts of heterologous endocrine glands can gradually induce resistance through the development of antihormones. However, it seemed unlikely that the body could become insensitive to its own hormones, since this type of resistance would be expected to interfere with the physiologic activity of endocrine glands. Still, the observation that partial hepatectomy sensitizes to the anesthetic effect of natural steroid hormones suggested that the liver does possess a mechanism for the inactivation of these compounds. The question arose whether this defensive activity could be stimulated by very large amounts of those substrates which the inactivating mechanism is designed to metabolize.

Experiments performed in rats to check this possibility revealed that following repeated massive overdosage with progesterone, desoxycorti-

costerone, or testosterone, the anesthetic effect of these hormones gradually diminishes. In fact, this type of resistance is not substrate specific, since pretreatment with any one of these natural steroids also induced resistance to the others [7].

Apparently, at near-physiologic dose levels, the natural steroids do not markedly activate this defense mechanism (a phenomenon which would interfere with their normal function), yet they may accelerate their own degradation more intensely when given in abnormally high and potentially pathogenic amounts. It is difficult to explain this dose dependence of the inactivating mechanism, and available data do not justify far-reaching speculations. However, it may be pertinent that at near-physiologic concentrations the steroid hormones circulate mainly as protein complexes, which are perhaps unable to reach the inactivating receptors; conversely, after sudden flooding of the body with very large amounts, a certain portion of the injected steroid may enter the inactivating sites (e.g., the SER) before being thus protected by coupling to large carrier molecules.

Still, even at physiologic concentrations, gonadal steroid hormones do appear to affect drug sensitivity to some extent, as shown by sex differences in the susceptibility to various intoxications. It remained to be seen, however, whether this physiologic difference in sensitivity and the induction of resistance by excessive amounts of exogenous steroids depend upon the same mechanism.

3. SEX HORMONES INFLUENCE RESISTANCE TO MANY DRUGS

Several earlier observations had suggested that sex differences in drug sensitivity are due to steroid hormones produced by the gonads and not to some inherent genetically determined resistance factors in the peripheral cells themselves. It would be beyond the scope of this outline to review the voluminous pertinent literature in detail; suffice it to say that although some sex difference in susceptibility to certain drugs may persist after gonadectomy [8, 9], most investigators agree that this is uncommon. Thus, adult male rats are less sensitive to barbiturates than females, but this difference disappears after gonadectomy, and the resistance characteristic of intact males can be induced in females, or gonadectomized rats of either sex, by treatment with testosterone and related compounds [10–13].

Our observations on the induction of resistance to the anesthetic effect

of steroid hormones by pretreatment with massive doses of the same or even of other steroids led us to reexamine the physiologic implications of this problem. In particular, we wanted to know whether the amount of testoids produced by the gonads themselves would suffice to raise resistance against intoxication with massive doses of other steroids. Experiments designed to clarify this point also promised to show whether near-physiologic quantities of steroids can protect, not only against substances foreign to the body, but also against compounds normally produced by the organism.

Here again, anesthesia proved to be a suitable indicator of a systemic intoxication with natural steroid hormones. Female rats were found to be more sensitive than males to progesterone anesthesia, but this sex difference became obvious only after maturity. We concluded that "normal endocrine activity of the testis is largely, if not entirely, responsible for this comparative resistance of the male, since castration increases sensitivity in males but is without effect in female rats. Conversely, the resistance of castrate males and females may be raised by methyltestosterone administration" [14, 15].

This sex-hormone-dependent resistance was subsequently confirmed in other types of intoxications with natural products. It was noted that female rats are much more sensitive than males to the production of hyalinosis and hypertension by desoxycorticosterone after conditioning by unilateral nephrectomy and an excess of NaCl [16]. Furthermore, the lesions thus produced could be inhibited by methyltestosterone [17].

A similar sex difference in susceptibility has also been noted with regard to the cardiovascular calcification elicited in rats by overdosage with dihydrotachysterol (DHT). This form of calcinosis was aggravated by orchidectomy and, hence, we concluded that "some testicular factor exerts a protective effect against this type of intoxication" [18].

The fact that partial hepatectomy increases sensitivity to drugs (e.g., tribromoethanol) and steroid hormones (e.g., desoxycorticosterone, progesterone) suggested that the liver plays an important part in defense against certain intoxications; however, it remained to be seen whether here the resistance depends upon an actual degradation of the toxic compounds within the liver or upon extrahepatic detoxifying mechanisms merely controlled by chemical compounds that the liver produces.

4. INDUCED HEPATIC DETOXIFICATION OF DRUGS CAN BE DEMONSTRATED IN VITRO BY INCUBATION OF THE SUBSTRATE WITH LIVER TISSUE

Soon after it had been observed that partial hepatectomy increases sensitivity to the anesthetic action of steroid hormones, Zondek, Sulman, and Sklow [19] showed that both estrone and stilbestrol can be inactivated by rat liver pulp in vitro, and that "in rats treated with large amounts of stilbestrol, the capacity of the liver to inactivate stilbestrol is increased."

Subsequently a group of investigators at our school undertook an extensive study of the relationship between sex differences in barbiturate resistance and the inactivation of barbiturates by liver tissue. They noted that pentobarbital anesthesia lasts much longer in female than in male rats, and that the high resistance of the male is abolished by castration but restored to normal by testosterone. In ovariectomized rats, estradiol was virtually ineffective, but testosterone raised resistance to the male level. All these in vivo effects were found to run parallel with the pentobarbital detoxifying power of hepatic tissue in vitro [20]. Liver homogenates of intact adult male rats destroyed pentobarbital in vitro more rapidly than those of castrate males. Furthermore, pretreatment of the castrates with testosterone enhanced the detoxification process, whereas estradiol pretreatment had an opposite effect [12].

5. INDUCED HEPATIC DETOXIFICATION OF DRUGS IN VITRO DEPENDS LARGELY UPON THE PRODUCTION OF APPROPRIATE MICROSOMAL ENZYMES

The next important point in the elucidation of the hepatic drug metabolizing mechanism was the demonstration of its dependence upon certain microsomal enzymes. It was found that the liver of the mouse and rat possesses an enzyme system which N-demethylates 3-methyl-4-monomethylaminoazobenzene. The activity of this system depends upon the diet, being highest on aged or otherwise treated animal products, such as an old cholesterol preparation, liver extracts, and peptones. A variety of pure sterols were inactive but could be activated by peroxidation [21].

There followed a large number of publications suggesting that the induction of this type of resistance depends upon corticoid [22–27], folliculoid [28], testoid, or anabolic [29–35] activity.

It was not until quite recently that the independence of this enzyme-inducing capacity from all known steroid hormone actions could be demonstrated [1]. It was found that in the rat, pretreatment with a variety of catatoxic steroids, such as spironolactone, norbolethone, and ethyl-

estrenol, increases the oxidation of pentobarbital by hepatic microsomes and enhances its disappearance from the blood proportionally to their ability to shorten the depth of anesthesia in vivo [36]. Norbolethone and ethylestrenol possess strong anabolic properties but little or no antimineralocorticoid effect, whereas spironolactone is a strong antimineralocorticoid devoid of anabolic actions. Since none of these steroids exhibits glucocorticoid, mineralocorticoid, or folliculoid effects, the catatoxic enzyme-inducing property appears to be independent of the latter.

6. PROLIFERATION OF SER IN HEPATOCYTES IS CHARACTERISTIC IN ENZYME INDUCTION

A number of investigators believe that the induction of hepatic microsomal enzymes by various drugs and steroids is associated with a marked proliferation of the SER in the cells of the liver [33, 37–41]. This effect is also independent of the known steroid hormone actions, and can be demonstrated in rats after treatment with such typical catatoxic steroids as spironolactone [42] or norbolethone [43]. However, it remains to be seen whether the proliferation of the SER is always associated with catatoxic activity.

7. NUMEROUS APPARENTLY QUITE UNRELATED PATHOLOGIC CHANGES ARE PREVENTABLE BY VARIOUS TYPES OF STEROIDS IN INTACT ANIMALS

One of the most characteristic features of catatoxic steroids is that they raise resistance to various toxic agents above the normal level maintained by the corticoids. Thus, unlike the latter, they do not merely substitute for insufficient adrenocortical function but increase tolerance to many injurious agents above that assured by the function of the adrenal cortex.

Many catatoxic steroids undoubtedly exert their protective effect through the induction of hepatic microsomal enzymes; hence, in vitro determination of the latter has often been used to assess the potency of drug-metabolizing agents. While such tests are perfectly appropriate for the assay of enzyme induction, they do not provide definite evidence of catatoxic potency. The induction of a specific enzyme activity (e.g., an oxidase or demethylase system) in the microsomal fraction of hepatic homogenates does not necessarily prove that this change has defensive value in the intact living animal; it tells us even less about the kind of morbid lesion that could be prevented. Indeed, changes in drug metabolism

caused by such inducible enzymes may even increase, or qualitatively alter the toxicity of drugs, depending upon the kind of abnormal metabolite produced. Furthermore, certain catatoxic effects may result from the action of steroids upon receptors other than the hepatic microsomes.

Hence, the most convincing direct evidence of protective activity has been obtained by testing the ability of steroids to prevent the production of well-characterized functional or structural changes by specific pathogens in vivo. Such bioassays are eminently suited for the screening of potentially catatoxic steroids and for determining the spectrum of their protective effect against various pathogens, yet they provide little or no information concerning syntoxic or catatoxic nature of the mechanisms involved. In other words, these in vivo tests tell us only which steroid can protect against which pathogen; but thereby, they provide models for further studies planned to identify the underlying mechanisms and the possible physiologic and pharmacologic implications of the resistance phenomena thus detected.

Numerous observations have shown that various catatoxic steroids can prevent death in intact rats treated with normally fatal doses of diverse toxic agents, such as nicotine, cinchophen, ethion, and several phosphothioate insecticides. However, here we shall discuss only the prevention of certain well-characterized functional or structural changes, such as anesthesia, convulsions, muscular paralysis, intestinal ulcers conducive to peritonitis, adrenal nectrosis, calcification of soft tissues, the "progeria-like syndrome," and myocardial necroses.

Pretreatment with norbolethone protects the rat against anesthesia induced by progesterone, desoxycorticosterone, pregnanedione, dehydroepiandrosterone, testosterone, diethylstilbestrol, and methyprylon. On the other hand it does not significantly alter the sedative effects of urethan, diazepam, chlorpromazine, reserpine, phenoxybenzamine, chloral hydrate, KBr, or $MgCl_2$ [44]. The antimineralocorticoid compound, spironolactone, exerts a similar protective effect against these same anesthetics [45]. Another highly potent mineralocorticoid, SC-11927, has not been tested against as many sedatives, but it is highly effective in preventing progesterone and pentobarbital narcosis [46]. All anabolics were highly potent, among numerous steroids screened for their ability to inhibit progesterone and pentobarbital anesthesia, but so was spironolactone, which is completely devoid of anabolic activity. "This inhibition of

anesthesia is assumed to represent a special instance of the catatoxic effect which appears to be a property of certain steroids, independent of their classic hormonal actions" [47].

The violent and often fatal convulsions produced by heavy overdosage with pentylenetetrazol in the rat can be prevented by large doses of progesterone or desoxycorticosterone. Conversely, the anesthetic effect of overdosage with these steroids is abolished by pentylenetetrazol [48]. These antagonisms had been known for a long time but they are probably unrelated to the catatoxic effect, since they occur immediately after injection before enzyme induction could have taken place; also, desoxycorticosterone has subsequently been shown to possess no, and progesterone only weak, catatoxic potency, when tested by modern techniques. It may be presumed that in the acute experiments the anesthetic and analeptic effects mutually blocked each other by a specific pharmacologic antagonism. On the other hand, more recent observations have shown that in rats pretreated with typical catatoxic steroids which have no anesthetic effect (e.g., ethylestrenol, spironolactone, SC-11927, norbolethone), the convulsive effects of pentamethylenetetrazol and even picrotoxin are likewise inhibited [Selye, unpublished].

The convulsions induced in rats by sublethal or lethal overdosage with digitoxin are particularly easily prevented by pretreatment with anabolic steroids; however, they are also blocked by spironolactone, and hence this protective effect was likewise ascribed to the catatoxic rather than to the anabolic action of the steroids [49, 50]. This type of prophylaxis is also effective against other cardiac glycosides. Thus, both spironolactone and norbolethone protect the rat against the convulsive and fatal actions of heavy overdosage with gitalin, proscillaridin, digoxin, and digitalin. Curiously, under identical conditions the corresponding effects of strophanthin K, ouabain, and digitoxigenin are not prevented [51].

The muscular paralysis induced in rats by large doses of mephenesin is inhibited by ethylestrenol, spironolactone, and norbolethone, that is to say, by steroids which appear to have in common only their catatoxic effect [Selye, unpublished].

In the rat, repeated subcutaneous injections of large doses of indomethacin produce multiple, often perforating jejunal ulcers which, under normal conditions, invariably result in fatal, generalized peritonitis. These changes are completely prevented by pretreatment with typical catatoxic

steroids, such as ethylestrenol, SC-11927, spironolactone, norbolethone, or oxandrolone. However, in this respect, progesterone and prednisolone also exhibit a definite, though less powerful, protective effect, whereas triamcinolone and hydroxydione are inactive [52, 53].

The inhibition of indomethacin-induced perforating fatal intestinal ulcers is one of the most dramatic and reliable indicators of catatoxic potency; it is regularly demonstrable even in rats given several times the normally fatal amount of indomethacin. This test has also been useful in demonstrating that the protection offered by chronic treatment with catatoxic steroids persists for long periods. We have observed that rats, given daily subcutaneous injections of fatal amounts of indomethacin, are maintained in excellent health for periods of six weeks or more by the concurrent daily oral administration of spironolactone, norbolethone, or oxandrolone. It appears that the catatoxic steroids do not induce resistance against themselves (as judged by their long-continued protective action), while they are highly efficacious in abolishing the toxicity of the ulcerogenic drug. It is especially noteworthy that among the two potent glucocorticoids of this series prednisolone is, whereas triamcinolone is not, endowed with protective properties. This fact cannot be ascribed to quantitative differences in glucocorticoid activity, since triamcinolone is totally devoid of indomethacin-antagonizing potency at all dose levels, from those causing a just-detectable thymus atrophy to fatal amounts.

Dimethylbenz(a)anthracene (DMBA) causes hemorrhagic adrenal necrosis in the rat, and this effect is likewise prevented by the catatoxic steroid spironolactone [54]. It had been shown previously that these adrenal lesions, unlike those elicited by the DMBA-metabolite 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-MBA), are inhibited by partial hepatectomy. Therefore it was assumed that DMBA is active only after having been metabolized in the liver to 7-OHM-MBA, whose effect is independent of hepatic function. The question arose whether spironolactone abolishes the adrenotoxic effect of DMBA by preventing its hydroxylation. However, apparently this is not the case, since this steroid protects the adrenals also against the damage caused by 7-OHM-MBA [55].

A considerable amount of work has been done on the prevention by catatoxic steroids of soft tissue calcification and the "progeria-like syndrome" (catabolism, wrinkling of the skin, gonadal atrophy, kyphosis,

calcifying arteriosclerosis, skeletal and dental lesions similar to those seen in senility) that can be elicited in rats by overdosage with dihydrotachysterol (DHT) and related compounds. It was shown as early as 1957 that the cardiovascular calcification and nephrocalcinosis produced by DHT in rats are aggravated by estradiol, cortisol, ACTH, and thyroxine, but inhibited by methyltestosterone [56].

Subsequently it could be demonstrated that the myocarditis, with calcification of cardiac muscle and coronary arteries, as well as the nephrocalcinosis produced in rats by combined treatment with DHT + Na_2HPO_4, are prevented by SC-11927 [57]. It was already known at that time that SC-11927 is a potent antimineralocorticoid, but its anti-DHT effect was not likely to depend upon this property; recent experiments suggest that it is more probably related to the catatoxic action common to methyltestosterone and SC-11927.

Among thirty-six steroids examined for their ability to protect the rat against the progeria-like syndrome elicited by chronic treatment with DHT, the strongest anabolic steroids (e.g., norbolethone, SC-7924, and fluoxymesterone) proved to be most efficacious [58]. These same steroids are also especially catatoxic in many other tests.

The generalized tissue calcinosis produced by a single high dose of DHT can be inhibited (in approximately decreasing order of activity) by pretreatment with SC-11927, norbolethone, oxandrolone, ethylestrenol, methylandrostenediol (MAD), methyltestosterone, prednisone, and spironolactone. A trace of inhibition was apparently also obtained by progesterone, prednisolone, and triamcinolone, but with the glucocorticoids, mortality due to intercurrent infections was so high that the mildness of calcification may have been due to the premature death of the animals. DOC had no protective action [59].

These findings again suggested that the anti-DHT effect of the steroids is not due to antimineralocorticoid, anabolic, or any other classic steroid hormone action but to their catatoxic potency.

Finally, several types of infarctoid myocardial necroses could be prevented by catatoxic steroids. In the rat, spironolactone inhibits the normally fatal extensive necroses of the cardiac muscle produced by certain corticoids given in combination with bisodium phosphate, excess fat intake, or exposure to stress [60]. The electrolyte steroid cardiopathy with necrosis (ESCN), produced by fluorocortisol or digitoxin in com-

bination with Na_2HPO_4 + corn oil, can also be prevented by such typical catatoxic steroids as spironolactone, ethylestrenol, norbolethone, and SC-11927. In this respect, oxandrolone, desoxycorticosterone, progesterone, prednisolone, and triamcinolone had little or no protective value. The ESCN produced by fluorocortisol + Na_2HPO_4 + restraint, or fluorocortisol + $NaClO_4$ + corn oil, proved to be much more resistant to prophylaxis; it was markedly diminished in intensity only by SC-11927 and spironolactone [61, 62]. Presumably in these experiments the protective action of the catatoxic steroids may be ascribed to their ability to inactivate fluorocortisol and related mineraloglucocorticoids which play an indispensable part in the production of this type of myocardial injury.

8. RECOGNITION OF CATATOXIC STEROIDS AS AN INDEPENDENT PHARMACOLOGIC GROUP

It is evident from this review that examples of protection by steroids against several injurious agents have been known for many years. Some of these were ascribed to specific pharmacologic antagonisms (e.g., inhibition of mineralocorticoid effects by antimineralocorticoids or of catabolics by anabolics). The nonspecific resistance obtained in adrenalectomized animals by corticoids appeared to be related to the "life-maintaining effect" which combats stress, no matter how produced. However, this latter action merely restores normal resistance in the absence of the adrenals, and hence it is essentially a substitution therapy. The antiphlogistic effect of exogenous glucocorticoids goes beyond this, since it raises resistance to various inflammatory irritants more effectively than can the normal adrenal cortex through its secretory activity. However, these effects are not directed against toxic agents; they only suppress a particular somatic response (e.g., catabolism, hypotension, inflammation) which may be harmful or beneficial, depending upon the circumstances.

Soon after the description of the "granuloma pouch technique" for the study of inflammation, it was noted that, in rats, the intense exudative inflammation elicited by croton oil, following its introduction into a subcutaneous air sac, can be totally suppressed by glucocorticoids (such as cortisol) without the destruction of the irritant. Even after a sojourn of two weeks in the pouch of the cortisol-treated animal in which it caused no inflammation, the croton oil retained its ability to produce severe inflammatory phenomena upon injection into the paw of unpretreated

rats. Here, the hormone acted by a syntoxic effect, that is, by inhibiting the reactivity of the tissues rather than by destroying the irritant [63].

The characteristic feature of catatoxic steroids is their ability to inactivate numerous toxic agents. This mechanism of action was not immediately evident, but it had long been realized that what we now call the catatoxic property of steroids differs qualitatively from their other protective actions. It is distinct from their most nonspecific (essentially "antistress") effect, which merely maintains the resistance of adrenalectomized animals at about normal, as well as from the highly specific pharmacologic antagonisms through which steroids can augment tolerance far above the norm, but only to a few compounds which happen to possess actions opposite to their own.

Later, when evidence on drug-metabolizing hepatic microsomal enzyme induction began to accumulate, the question arose whether this effect might not explain some of the catatoxic actions of steroids and other hormones. Of course it would be an oversimplification to say that an increase in resistance is the necessary consequence of such enzyme induction; depending upon circumstances, hepatic enzymes may regulate growth metabolism or reproduction without significantly affecting systemic resistance. Innumerable factors influence the physiologic consequences of a particular enzyme system, and hence the in vitro demonstration of its induction can furnish, at best, only presumptive evidence concerning its protective effect in the intact animal. Steroid hormones activate enzyme systems in many tissues, but even if specific hepatic microsomal enzyme induction is proven, a catatoxic effect cannot be ascertained unless it results in a relatively nonspecific increase in resistance under in vivo conditions. This type of tolerance, though not predictable with certainty, may be expected when multipurpose drug-metabolizing enzymes (e.g., oxygenases, demethylases) are activated. The induction of such enzymes has frequently been demonstrated in the microsomal fraction of hepatic homogenates; in some cases it was even possible to demonstrate its defensive value in vivo, especially when barbiturates, muscle relaxants, or insecticides were used as substrates. However, to map out the entire scope of such enzyme induction by natural compounds in pathology, large-scale screening on different disease models in vivo will be required. The first efforts along these lines have been reported in the

preceding pages. They have already demonstrated that many morbid lesions, widely differing both qualitatively and in their organ distribution, can be thus prevented by pretreatment with steroids. It is still uncertain whether all of these prophylactic effects depend upon hepatic microsomal enzyme induction, but in many cases this appears to be the case.

These considerations have guided the attempt of this review to correlate our earlier observations on the increase in drug resistance, after partial hepatectomy and the protective effect of certain steroids, with the more recent observations on the induction of hepatic microsomal enzymes. The latter effect has been studied extensively in many laboratories, using corticoid, folliculoid, luteoid, testoid, or anabolic steroids. However, the interpretation of the results was handicapped by the a priori assumption that the observed enzyme-inducing effects are necessarily the subordinate, secondary consequences of the predominant hormonal action of whichever class of compounds was employed.

The findings surveyed here strongly suggest that this is not the case, since the same type of catatoxic activity is demonstrable in steroids devoid of any one among these classic hormonal actions. Indeed, the most potent catatoxic steroids, among all those investigated so far, are two antimineralocorticoids (spironolactone and SC-11927), which do not exhibit significant degrees of any other pharmacologic activity, and an anabolic agent (ethylestrenol), which is virtually devoid of antimineralocorticoid potency. It might have been thought that some of these steroids act through specific pharmacologic antagonisms against different compounds, but this is not so, since the same steroids protect the rat against any toxic agent whose effect is subject to this type of inhibition (e.g., digitoxin, indomethacin, DHT, barbiturate, or steroid anesthesia).

Therefore, we must conclude that the catatoxic effect is just as much a separate, independent property of steroids as the other classic actions (e.g., corticoid, testoid, folliculoid, luteoid, anesthetic) which are likewise not subordinate to each other. It is of practical importance to realize this fact, since it implies that we need not limit the search for ever-more active catatoxic steroids to compounds exhibiting one or the other hormonal action which has been claimed to be responsible for drug-metabolizing enzyme induction. Indeed, there is no reason to doubt that such a protective effect may be found among steroids possessing none of the known hormonal effects.

Outlook

In appraising the scope of research on catatoxic compounds, it should be emphasized that their protective action is not limited to the detoxification of drugs foreign to the body. It had been thought at first that "the microsomal enzyme systems in liver may be there just to 'detoxicate' foreign compounds . . . we have found no normal substrate which is metabolized by the drug enzyme systems in microsomes. If we think of the microsomes as particles with a membrane which will, ordinarily, pass non-polar compounds but not polar compounds, we have a plausible picture of the way the body protects its essential substrates from wasteful metabolism due to the non-specific microsomal enzymes" [64]. Indeed the most recent monograph on drug-metabolizing enzymes refers to them as "xenobiotics" (i.e., compounds that deal with foreign materials) [65]. Yet, as shown by the many examples listed in this review, both the catatoxic effect in vivo and microsomal enzyme induction demonstrable in vitro can be induced against a variety of naturally occurring substrates, particularly steroid hormones and vitamin-D derivatives. It is not unreasonable, therefore, to think of the catatoxic steroid system as one capable of protecting the organism not only against intoxications with exogenous poisons, but also against toxic substances that may arise within the body and be the cause of disease. It will be a fascinating new field of research to elucidate the mechanisms through which the body can prevent the enzymatic destruction of useful metabolites when they are present in physiologic amounts.

We have no evidence as yet that the endogenous secretion of catatoxic steroids can be increased as a defensive measure in response to need, just as corticoid production rises under conditions of stress. However, many of the catatoxic steroids are natural products that might thus be made available.

Up to now research on nonspecific defense reactions against injury was concerned mainly with the activation of the sympathetic nervous system (Canon's Emergency Reaction) and of the hypothalamus-pituitary-adrenocortical response to stress (the General Adaptation Syndrome). Neither of these responses attacks the pathogen; they merely adapt the body to its actions. It appears that in the future we should give equal attention to the catatoxic hormone system, which may include even

nonsteroidal enzyme inducers. Its activity is undoubtedly conditioned by, and correlated with, nervous and adrenocortical reactions to stress; yet it differs from these, both in its mechanism of action, which is designed to destroy the pathogen, and in the spectrum of protection offered.

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