

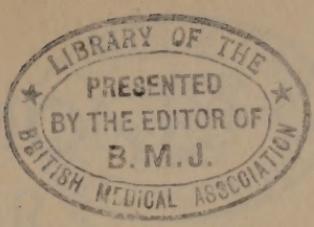
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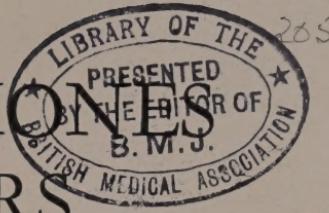
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STEROID HORMONES AND TUMORS



205

TUMORIGENIC AND ANTITUMORIGENIC
ACTIONS OF STEROID HORMONES
AND THE STEROID HOMEOSTASIS

Experimental Aspects

BY

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PREFACE

The problem of steroid hormones and cancer, or tumors in general, has become in the course of a couple of years one of burning actuality. Clinical progress, though still in a very limited field, has been achieved, and is likely to be achieved in the future only thanks to close contact with experimental research. Endocrinological problems which hitherto seemed to be of interest only for the research worker may suddenly acquire real practical importance. As to this, things in this special domain of pathology are indeed not different from what is going on in modern human life in general.

Since 1936 the author and his associates have published a series of papers dealing with the production and inhibition of tumoral growth by steroids. Many of our findings have been described only in the Thesis of M.D. of Universidad de Chile, or have not been described at all. Thus it was thought that a monograph should be written to bring together the work of fifteen years in a condensed form, and to follow in the exposition a stricter sequence than possible when publishing experimental results as they originate during research work continued for years. The same plan of exposition has been adopted in three lectures delivered in October and November 1946 in the Royal College of Surgeons of England, under the auspices of The British Council, and in different Faculties of Medicine in England, France and Switzerland.

Comparative summaries of the work done since 1932 by the authorities on estrogen-induced tumors also have been included. Through the references the reader will be able to make contact with the whole field. The book may thus be of use both to the research student and the clinician as an introduction into a fascinating chapter of modern research on tumors. Indeed, mention of some important findings and papers has been probably, but unwillingly, omitted; I should like to lay special stress on my statement that omission does not signify lack of appreciation on my part for the work done by the respective authorities.

Use has been made in this book of new words as "tumorigenesis," "tumorigenic" and "antitumorigenic," so as to include problems which refer to the origin, prevention and therapy of all kinds of neoplasms, not only malignant carcinoma and sarcoma, but also benign tumors, spontaneous or experimental, and even those processes which are on the border line between localized hyperplasia and neoplastic growth in the traditional acceptation. On philological grounds "tumorigenesis" was declared to be inadmissible on account of being half Latin and half Greek, and "blastomatogenic" was proposed instead (Woglom, 1942). But should these philological grounds be really relevant the whole medical terminology should be overthrown:

immunology, serology, hydrocarbons, and so on, are all half Latin, half Greek. "Blastomatogenic" has also the disadvantage of being too difficult to pronounce, especially when in combination with a prefix as for instance in "anti-blastomatogenic." Subsequently I had the satisfaction that "tumorigenesis" was adopted by several leading authorities.

Though the book has been written by me it is the result of a *collective* effort.

In our work with steroids I have enjoyed constant and indefatigable help on the part of Dr. *Carl Miescher*, Director of Ciba Pharmaceutical Products in Basle, Switzerland. Dr. *Miescher's* researches on the chemistry of steroids have been fundamental, and we had the privilege of amply profiting by them. This collaboration not tinted with any economic interest has been decisive for the progress of our work in the last twelve years.

My comprehension, in our own field of research, has been greatly stimulated by Dr. *Peyton Rous'* discovery of two fundamentally different evolutional phases of neoplastic growth induced by hydrocarbons, the phases of "initiation" and of "promotion". I should like to render my homage to Dr. *Rous* for this ingenious experimental work remarkable both for the theoretical reach of the results and the simplicity of the technical means used.

It gives me also pleasure to mention at this place my distinguished friend and colleague Dr. *Eduardo Cruz-Coke* with whom I had many stimulating conversations in the course of years on the subject of cancer and hormones. It is also to the care of Dr. *Cruz-Coke*, then Minister of Health, that I owe the creation of my Department.

Our results rely on observations made on several thousands of animals and rendered possible only thanks to the collaboration of younger workers. I am deeply indebted especially to those associates who since 1937 have stayed for several years with me as Drs. *R. Iglesias*, transitorily Research Associate at the Ochsner Medical Foundation, Tulane University, New Orleans; *L. Vargas*, now Professor of Pathological Physiology at the Catholic University of Chile; Drs. *S. Bruzzone*, *F. Fuenzalida*, and *A. Riesco* of this Department. Several of them have read the manuscript and have made valuable suggestions. Drs. *Iglesias* and *Bruzzone* were helpful also in completing the references. Dr. *Fuenzalida* was so kind as to control the chemical formula.

Those who joined us from other countries, also may be especially mentioned as Drs. *Christiane Dosne* from Canada, *Raul Franco de Mello* from Brazil, *O. Koref* and *I. Szabó* now of this country, and *J. Schwarz* now of the Jewish Hospital in Cincinnati. Many others—Chileans and students of the School of Medicine of Universidad de Chile coming from various Spanish-American countries—have collaborated with the author and his associates.

Our research has been greatly helped by the Rockefeller Foundation;

The Jane Coffin Childs Memorial Fund for Medical Research; and the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

The Charles L. Mayer Cancer Award administered by the National Science Fund of the National Academy of Sciences of the United States has been given to the author in 1944 for part of the work dealt with in the present book.

Collaboration offered by the scientific staff of chemical concerns has been essential. Considerable help was made available—in the course of many years—by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Summit, N. J., U. S. A.; by Dr. E. Schwenk of Schering Corporation, Bloomington, N. J.: Dr. A. S. Cook of Messrs. Ayerst, McKenna and Harrison of Montreal; Dr. Oliver Kamm of Messrs. Parke, Davis and Co.; Dr. F. Giral of Laboratorios Hormona in Mexico City; of Dr. H. Guggenheim of Messrs. Hoffmann-La Roche in Basle; Messrs. E. R. Squibb and Sons, New York; Dr. D. F. Robertson of Messrs. Merck & Co., Inc., Rahway, N. J.; Dr. M. Tausk of Messrs. Organon, Oss, Holland.

I am indebted to Dr. E. C. Dodds for samples of diethylstilbestrol and hexestrol put at my disposal soon after the discovery of these substances; and to Dr. E. C. Kendall for samples of dehydrocorticosterone and substance H.

I am greatly obliged to Mrs. Margaret Lipschutz for linguistic assistance; to Miss Dagmar Staden for valuable help with the manuscript and throughout the work; to Mrs. Julia Peña for technical assistance, to Miss Ana Contreras for microscopic work, to Mr. Siegfried Chaskel and Mrs. Daphne Pérez for photographic work.

I wish to express my thanks also to the Director General of Health of this country, Dr. Nacianceno Romero, for comprehensive help given to this Department in the course of many years.

Last but not least my thanks are due to The Williams & Wilkins Company who put extraordinary care in editing this book with great speed.

Acknowledgement for kind permission to quote and reproduce figures from the papers of the author and his students is due to the Editors of the following scientific journals: Cancer Research; Endocrinology; Proceedings of the Society for Experimental Biology and Medicine; Cold Spring Harbor Symposia of Experimental Biology; Science; Archives of Pathology; Revue Canadienne de Biologie; The University of California Press; The Lancet; Nature; Journal of Endocrinology; and The British Journal of Cancer.

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PART I.

The Tumorigenic Action of Steroids
and its Implication for the
Problem of Cancer

Chapter 1.

FUNDAMENTAL PROBLEMS OF TUMORIGENESIS AND CARCINOGENESIS

When referring to the tumorigenic action of steroids, that of estrogens is meant. Tumors induced experimentally by estrogens are too often noncancerous, and it may seem, at the first glance, a rather bold undertaking to deal with the implication of noncancerous tumoral reactions for the problem of cancer. But, as we shall see, an experimental analysis of the tumorigenic action of estrogens when logically developed to all its consequences, is liable to disclose before us, by and by, fundamental problems of neoplastic growth in general, including cancer. It must not be forgotten that real progress in the chemical treatment of cancer in the last years was due to the study of the manifold actions of steroids, as was the case with the hormone treatment of prostatic carcinoma introduced by Huggins.

“Fundamental” problems are always, so to say, “time conditioned,” i.e. they vary in the course of the research. At the actual moment one may tentatively resume the riddle of cancer in five partial problems:

First: The physiological growth substances of the body, or morphogenic factors, which induce normal or “*typical*” cellular proliferation, structural differentiation of the cells and organization of tissues; it is the problem of hormones, of induction and organizers and of their place in morphogenic processes.

Second: The conditions by which growth of a limited territory—a cell, or group of cells—is rendered “*atypical*” as to the rhythm of cellular proliferation, as to differentiation of cellular structure, and as to organization of tissues, a “*tumor*” finally arising out of the atypical growth.

Third: The *genetical* conditions referring (a) to an abnormal reactivity of a limited territory; (b) to a deviation in the production of physiological growth factors by which the latter become abnormal as to quantity, timing and chemical constitution; physiological growth factors acquiring both in case of (a) or (b) the quality of *tumorigenic* factors.

Fourth: *Exogenous* factors—including viruses—which under the given genetic conditions may act as, or enhance, tumorigens.

Fifth: The conditions by which the capacity of “*autonomous*” or “*neoplastic*” growth is acquired, that is to say the conditions by which

the faculty of continuous, illimitated, infiltrating growth, and the faculty to produce metastases, is irreversibly conferred to the cells which enter into atypical proliferation and differentiation.

Sixth: The problem of *antitumorigenic* substances or factors able to counteract the tumorigenic factors.

Those familiar with modern work in the field of carcinogenic hormones, hydrocarbons or viruses know that all these problems have been repeatedly the object of experimental research.

It is also evident that all the above mentioned fundamental problems of tumoral growth and cancer are intimately related to those of modern experimental embryology, or physiology of growth. And even more: when becoming aware of the above problems being part of the riddle of cancer, one fully understands that cancerous growth, as neoplastic growth in general, is but a problem of physiology of growth. We shall see in the Part III that even the problem of antitumorigenic factors—at the first glance only a practical problem of therapeutics—turns out, on the basis of experimental work, to be a problem of really fascinating interest in *physiology*.

Not only the worker in cancer research but likewise clinicians and social health workers will recognize that the mentioned fundamental problems are intimately related to the essentially practical tasks they have to deal with in cancer.

A discussion about the implication of the tumorigenic action of *steroids* for the problem of cancer cannot but refer to the same fundamental problems, with the special purpose to examine *whether, where and how steroids may interfere in the dynamics of cancer*. Such a discussion has to be based both on experimental and clinical research. Our examples shall be taken mostly from experimental research though reference shall be made also to human pathology.

The actual phase of research on steroids in neoplastic growth is not related to preceding work with cholesterol in this field. It has been shown by Roffo, as early as 25 years ago, that growing transplantable sarcoma and carcinoma of the rat contain much cholesterol, and that the latter increases in the blood serum of the animal with a growing tumor (see Roffo and Correa-Urquiza, 1942, p. 353-358); more recently these findings have been substantiated by the statement that the cholesterol content of transplantable rat hepatoma is the double of the cholesterol content of intact liver (work of Kishi, Fujiwara and Nakahara; quoted from Greenstein, 1947, p. 246). An increase of the cholesterol content of the skin after solar or ultraviolet irradiation, and production of transplantable malignant tumors by ultraviolet irradiation has been seen by many authorities (Roffo and Correa-Urquiza, 1942, p. 366-372). An increase of cholesterol has been found to take place also in the skin with advancing age and especially in individuals with "precancerous" lesions (Roffo and Correa-Urquiza, 1942, p. 386-401). Unfortunately bald theories on the implication of cholesterol in cancer have been forwarded on the basis of these statements. Various authorities claimed that induction of skin tumors by tar was enhanced by the

ingestion of cholesterol but proofs were extremely scanty (Roffo and Correa-Urquiza, p. 358-361). Roffo reported subsequently production of skin tumors by the ingestion of irradiated cholesterol and of gastric tumors by the ingestion of heated fat, as in cooking in which cholesterol deteriorates. The findings of Roffo with irradiated cholesterol have not been corroborated by other workers (summary of Cook and Kennaway, 1940, p. 403-405).* Indeed, it has been established that sarcomas can be induced in mice with commercial cholesterol; but the carcinogenic factor has not yet been isolated (Hieger, 1948, 1949, with refer. to former papers of the author). After Roffo, the carcinogenic action of heated fats has been reported by other workers also (Waterman, 1940; Peacock and Beck, 1948); but here again no knowledge apparently exists on the substances involved (Peacock, 1947). So far no carcinogenic substances have been isolated among the products of pyrolysis of cholesterol though several of the compounds obtained are intermediates in the transformation of cholesterol to methylcholanthrene (Falk et al., 1949). The fact that cholesterol increases in the skin after irradiation is no proof that the tumors elicited by the latter are due to this increase; no increase, but decrease of cholesterol to approximately 50 per cent of normal within a few days after a single painting with methylcholanthrene has been stated (Carruthers and Suntzeff, 1945).

No increase of urinary cholesterol has been found in cancer patients (Burchell et al., 1949).

Research on cholesterol in cancer has certainly been stimulating. But progress in work with the tumorigenic actions of steroids could not spring from the contradictory results with cholesterol; it sprung from the fundamental discoveries of Loeb and Lacassagne to which reference will be made below.

Much fundamental research has been done by various investigators since the early thirties with the administration of estrogens into rats, mice and other species, including monkeys (summaries of Lacassagne, 1939; Zuckerman, 1940; Burrows and Horning, 1947; Gardner, 1947a, 1947b).** The investigators established that atypical growth of the uterine mucosa was elicited when estrogens were administrated for a sufficient length of time. But others, and especially clinicians, raised the objection that the quantities administered were in most of this work very large and far from the quantitative potentialities of the ovary even under pathological conditions. There was, however, the classical statement of Loeb (Lathrop and Loeb, 1916; Loeb, 1919) that the mammary adenocarcinoma hereditary in the female of certain strains of mice is at its start dependent on the presence of the ovary; and there was more recently the most important discovery of Lacassagne (1932) that this mammary tumor can be made to appear precociously, and even in the male, by injection of estrogens, natural and synthetic (Robson and Bonser, 1938). But again, in hereditary carcinoma of the breast in mice,

* Different workers have also tried, unsuccessfully, to obtain carcinogens by extraction of the skin of patients with precancerous lesions of actinic origin (Mohs, 1948), or by ultraviolet irradiation of sterol fractions extracted from scalp hair or wool (Snapp et al. 1950).

** See also Allen, 1942; Dodds, 1944. Italian literature see Alfieri, 1938; Rondoni, 1946, p. 649-670; Picco, 1949. German literature see Von Wattenwyl (1944).

the role of the estrogen was shown to be different from that this hormone has in tumoral growth experimentally induced by its prolonged and continuous administration. Hormone production in mammary cancer strains may, indeed, be quantitatively different than in normal strains (Korteweg, 1948); but there is even in high-cancer strains always *rhythmic* action of estrogens, though the duration of the sex cycle varies somewhat according to the strain (Gardner, 1941b; Deringer et al., 1945; Armstrong, 1948; see also p. 98).

On the other hand as early as 1936 we were fortunate to give the experimental proof that a *hormonal imbalance* may be at the root of atypical, or tumoral, proliferation of the epithelia of the genital tract in the guinea pig. When by an operative interference on the ovary an experimental overthrow of the sexual cycle is produced, the animal's own estrogens may acquire the faculty to elicit atypical epithelial growth similar to that induced by the prolonged administration of the hormone (Lipschutz, 1936a, 1937, 1938; see Part III, ch. 18). This finding was to center our interest, from the beginning, on special problems to which, as it seems, less attention has been paid hitherto—both the *quantitative* and *timing* conditions under which estrogen becomes able to induce atypical growth. Experiments with the prolonged administration of estrogens in female guinea pigs were undertaken. In the course of these experiments fibroids disseminated in the whole abdominal cavity were found in almost all animals of the series (Lipschutz and Iglesias 1938; Iglesias 1938). We thought that tumors appearing so precisely under certain experimental conditions may serve as a test which would throw light on the very relevant question of how a steroid hormone essential for reproduction in the female and void of any toxic action when a single injection of large quantities is given, becomes so noxious for the body as to give origin to atypical epithelial and conjunctive growth. It was also thought that this would possibly allow studying of certain problems referring to tumor production in general. Accordingly, a scheme for further work was elaborated which at the onset included the following topics: The *exogenous* factors of the fibromatogenic action—the bearing of the quantities administered, of the timing conditions of administration, of the differential behavior of various natural estrogens, free and esterified, and of artificial estrogens; and the *endogenous* factors of fibromatogenic action—the bearing of age, sex and zoological species on the abdominal tumoral reaction, and the relevance of different abdominal organs for the production and localization of these fibroids.

The field of our research was widened when we tried to apply in our work on experimentally induced fibroids certain aspects of the antagonism of sex hormones we studied and propounded about twenty five years ago (see p. 125). As early as 1936 different workers showed that estrogen-induced atypi-

cal epithelial proliferation can be prevented by other steroids (see p. 160), and in our work steroids were found to be powerful antagonizers of the fibromatogenic action of estrogen.

Knowledge we acquired later on the quantitative and timing conditions of the fibromatogenic action of estrogens, on the differential antifibromatogenic activity of ovarian, testicular and cortical steroids, and on certain aspects of intrahepatic inactivation of fibromatogenic and antifibromatogenic steroids led us to the concept of an antitumoral autodefense in the body by the maintenance of a steroid balance—a kind of homeostasis, to use an expression of Cannon—of vital importance for the body, according to all probability.

Chapter 2.

ESTROGEN-INDUCED EXPERIMENTAL FIBROIDS

A. GENERAL

As early as fifteen years ago estrogen-induced fibromyomatous transformation of the uterine wall had been reported in the rabbit by Lacassagne (1935a). But Nelson working with guinea pigs was the first to establish that uterine fibroids may be induced by the prolonged administration of estrogens (Nelson, 1937, 1939). The same statement has been made independently by other workers (Lipschutz and Iglesias, 1938; Iglesias, 1938; Moricard and Cauchoix, 1938; Cauchoix, 1939). In the course of our experiments we had the good luck to establish two relevant new facts which were to open a field for interesting research: (1) the estrogen-induced fibroids are not only uterine but can be found so to speak *everywhere in the abdominal cavity*; and (2) under certain experimental conditions the abdominal fibroids are present in almost all animals of a series (Lipschutz and Iglesias, 1938). The finding of estrogen-induced uterine and extrauterine abdominal fibroids has been fully corroborated and extended by Sammartino and Herrera (1940), Von Wattenwyl (1941, 1944) and Mosinger (1946) who gave detailed and excellent descriptions of their results. The work of Bimes (1945), Ducuing (1946) and others also has to be mentioned (Woodruff, 1941; Perloff and Kurzrok, 1941; Marx, Glass and Shulman, 1942).

Let us take as an example a group of experiments in which estrogens have been administered to castrated female guinea pigs for about three to four months (Iglesias, 1938; Lipschutz and Iglesias, 1938; Lipschutz and Vargas, 1939a, 1941a). There were tumors of different sizes in the abdominal cavity and they were found, as already stated, so to speak everywhere: on the ventral and dorsal surface of the uterine horns; in the parametrium in

FIG. 3. Small pedunculated tumor near the parametrium. Apical tumor, or tumor of the mesosalpinx. 52 inj. of 80 µg of estrad. (benz.), 4 mo. Nat. size. (G.p. I.11).

FIG. 4. Subserous and parametrial tumors. A. Dorsal; B. Ventral. Right horn: chain of parametrial tumors almost ready to unite one with another. Left horn: two large parametrial tumors. 12 µg of estrone daily, 3 mo., from subcut. pellet. X 0.85. (G.p. LV.75).

FIG. 5. Large parametrial tumors. Enormous apical tumors. Left apical tumor reaches, and unites with, tumor of epiploon and pancreas; reaches also the spleen. 34 µg of estrad. (diprop.) per day, 108 days, from subcut. pellet. Nat. size. (G.p. LXI.16).

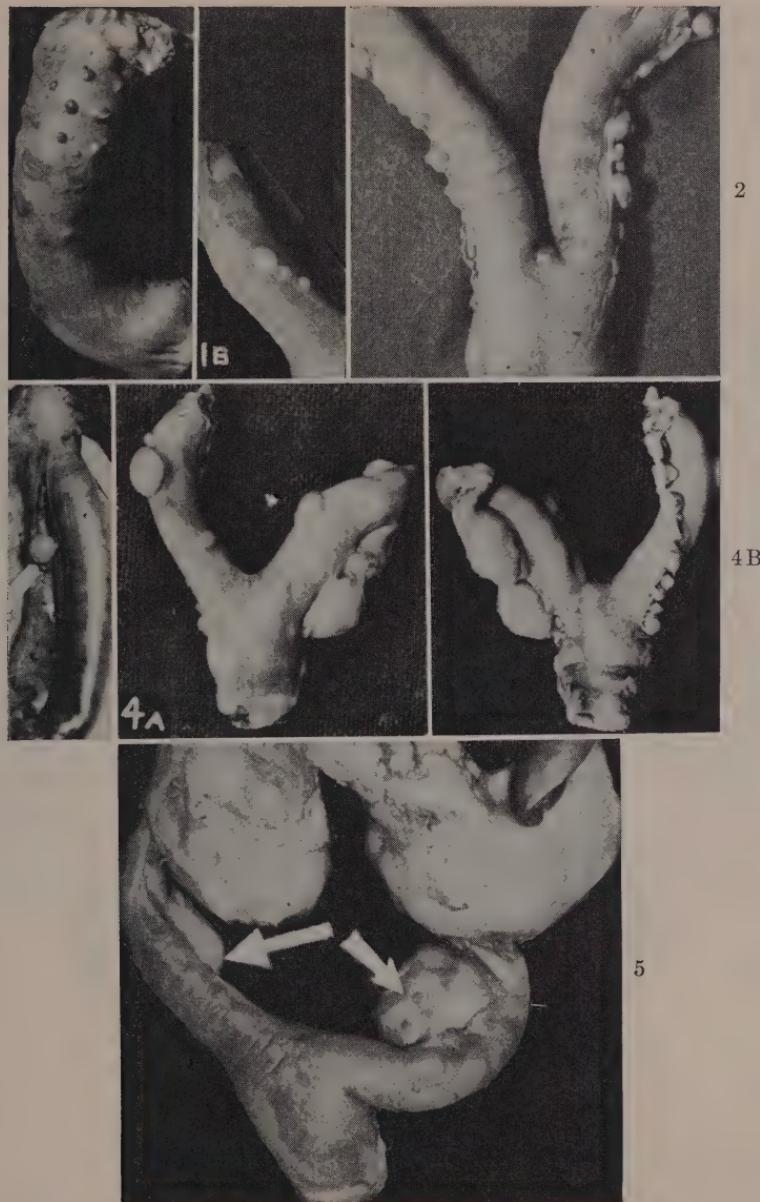


FIG. 1. Subserous nodules on the surface of the uterus. A. 52 inj. of 20 μ g of estrad. (benz.), 4 mo. Nat. size. (G.p. I.2). B. Chain of subserous uterine tumors following the line of the ventral muscular ridge. 40 inj. of 10 μ g of estrad. (capryl.), 108 days. Nat. size. (G.p. XII.71).

FIG. 2. Chain of small parametrial tumors. Small saddle tumor. 51 inj. of 80 μ g of estrad. (benz.), 4 mo. \times 1.1. (G.p. I.6).

intimate relation with the uterus and the parametric blood vessels; on the vaginal wall; on the mesosalpinx, and from there descending sometimes into

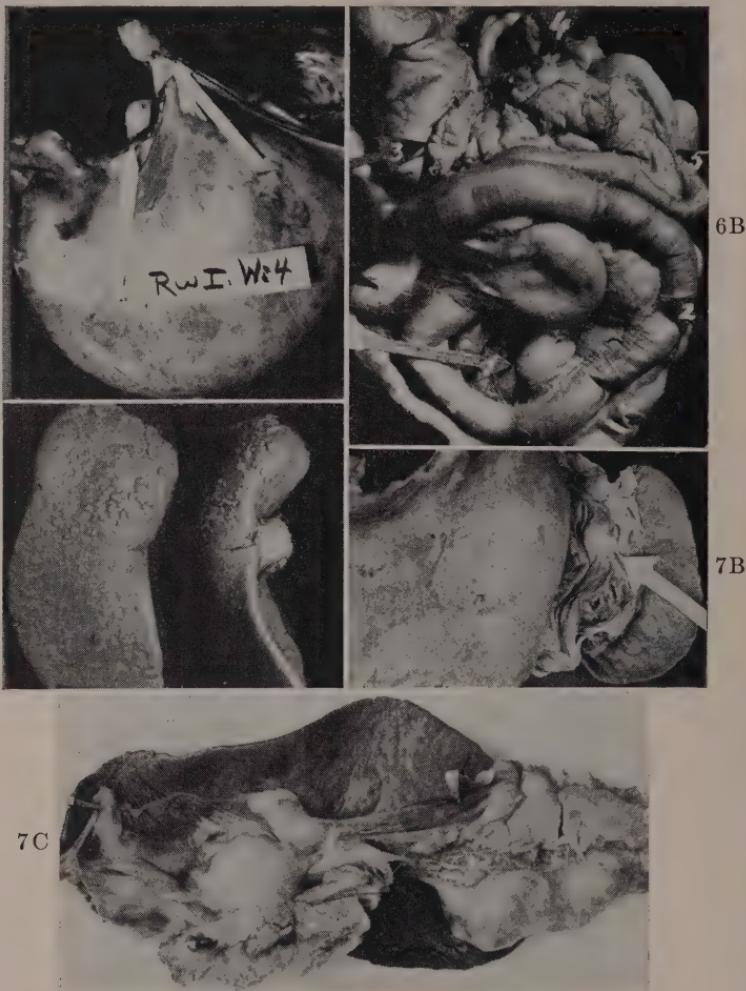


FIG. 6. Tumors of digestive tract. A. Tumor of cardias and small curvature. 53 inj. of 40 µg of estrad. (benz.), 4 mo. $\times 0.7$ (fem. G.p. I.4). B. Mesenteric tumors. Enormous tumor between arrows 3, 4 and 5; 1 and 2 point to smaller tumors. 51 inj. of 80 µg of estrad. (benz.) during 4 mo. Considerable quantity of liquid in the abdominal cavity probably due to compression of mesenteric veins. (fem. G.p. I.12).

FIG. 7. Tumors of the spleen. A. Dorsal and lateral view. Tumoral seed. Tumor in the hilus. 38 inj. of 2 µg of estrad. (capryl.), 88 days. $\times 1.4$ (fem. G.p. XXI.13). B. Tumor in the hilus. 38 inj. of 80 µg of estrad. (benz.), 88 days. $\times 0.8$ (fem. G.p. XV.11). C. Numerous tumors in the hilus. 40 inj. of 10 µg of estrad. (capryl.), 90 days. $\times 2.3$. (fem. G.p. XII.39).

the pelvis; in the mesentery beginning with the pylorus and duodenum down to the rectum; on the great and small curvature of the stomach; in the epiploic; near the cardias and the esophagus; on the surface of the pancreas and of the kidneys and rarely of the liver and gall bladder; on the urinary bladder; on the surface and in the hilium of the spleen; on the diaphragm and the abdominal wall. Figures 1 to 8 give an insight into the different localizations of the tumors and their variable size. Extrauterine fibroids are more frequent than uterine tumors and they may be present without there being



FIG. 8. Uterine and other abdominal tumors. Arrows 1, 2, 3, 5—tumors of the abdominal wall; arrow 4—large apical tumor; 6, 7, 8—uterine tumors; 9—mesenteric tumor; 10—very large mesenteric tumor. 73 inj. of 80 µg of estrad. (benz.), 6 mo. (G.p. I.24).

uterine ones. Full corroboration was given to these findings by all the workers.

Emphasis must be laid on the fact that incidence and the degree of the fibrous tumoral reaction varied greatly in different individuals and different batches obtained from dealers. We have not studied the question whether and how far these variations may be due to hereditary factors.

The incidence of uterine or extrauterine fibroids does not depend on the age of the animal (Lipschutz and Vargas, 1941d). In the work of Bruzzone (1943) estrogen was administered to newborn guinea pigs six to fourteen

days old and continued till the age of 79 to 87 days was reached; large uterine and extrauterine fibroids were produced in all the animals.*

Tumors were produced both in non-castrated and castrated females. Large uterine and extragenital tumors were observed in non-castrated females as early as 49 days after having begun administration of estrogens (Murillo, 1940). But most experiments have been made in castrated females,

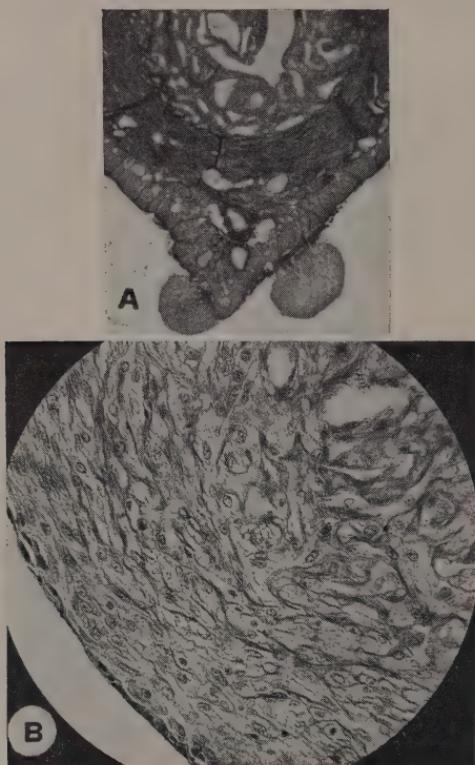


FIG. 9. Subserous uterine fibroids near the ventral muscular ridge. 40 inj. of 5 μ g of estrad. (benz.), 3 months (G.p. VI.96). A. Two small tumors. Cystic glandular hyperplasia and adenomatous polyps. $\times 10$. B. Fibroblasts in the peripheral belt of the tumor. Collagen fibers nearer to the center. $\times 200$.

so as to avoid any hormonal influence emanating from the ovary. In the first 2 months of estrogenic action the fibrous response was seemingly somewhat diminished when the ovaries were left in the body; but no ovarian influence

* Bruzzone made the interesting statement that in these young animals receiving estrogen the fibrous rudiment of the omphalo-mesenteric veins became persistent, thickened and sometimes adorned with small fibroids.

or inhibition was manifest when experiments lasted longer than 2 months (Lipschutz, Murillo et al., 1939). This was also the case in the work of Von Wattenwyl (1944; see also Sammartino y Herrera, 1940).

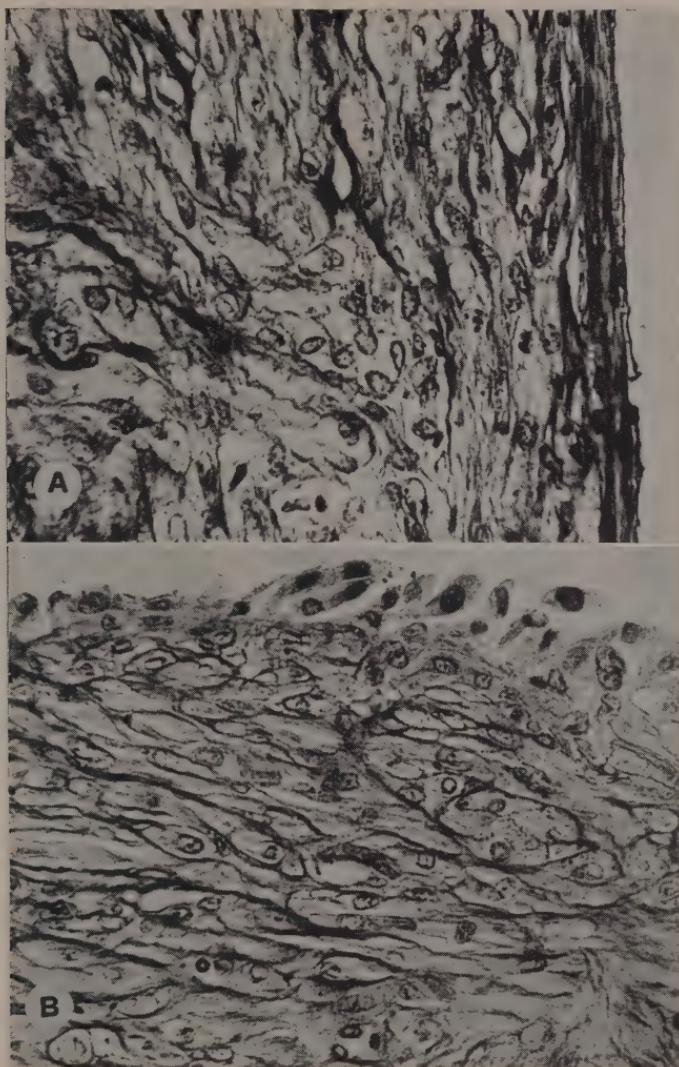


FIG. 10. Tumor between spleen and pancreas. A. 49 inj. of 80 μg of estrad. (benz.), 122 days. Peripheral belt of fibroblasts covered with several layers of flattened cells. $\times 400$. (fem. G.p. II.15). B. 43 inj. of 100 μg of stilbestrol, 105 days. Cells rich in cytoplasm on the surface of the tumor. Collagen fibers separating the fibroblasts of the peripheral belt. $\times 400$. (fem. G.p. IISt.2).

B. MICROSCOPIC STRUCTURE

When studying the structure of these abdominal tumors many interesting and varied aspects have been discovered.

The periphery of the tumor is built up by cells which remind one of fibro-

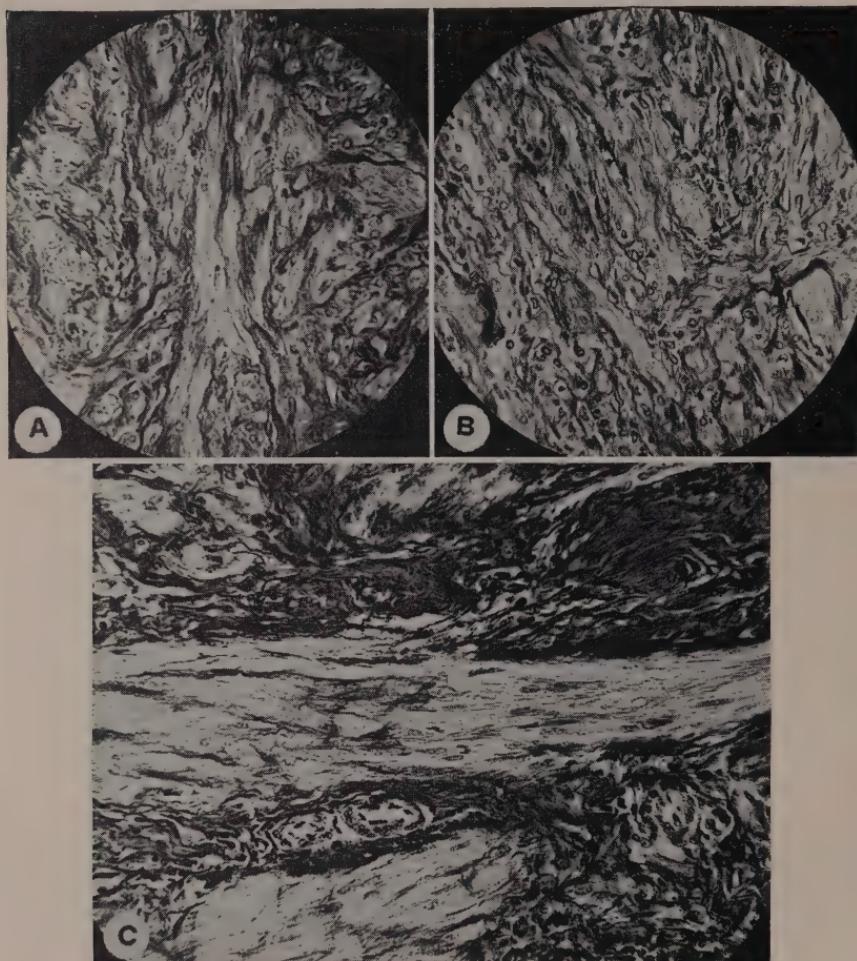


FIG. 11. Comparative structure of various tumors. A. Tumor of the hilus of the spleen in male. 43 inj. of 80 µg of estrad. (capryl.), 101 days. Abundance of smooth muscle fibers. $\times 200$. (G.p. VIII.1). B. Retrorenal tumor in male. 36 inj. of 80 µg of estrad. (capryl.), 87 days. Same tumor as fig. 22. Most probably smooth muscle fibers surrounded by thick collagen fibers. $\times 200$. (G.p. VIII.6). C. Fibromyoma between spleen and pancreas in female. 61 inj. of 80 µg estrad. (benz.), 142 days. Thick bundles of smooth muscle fibers. $\times 200$. (G.p. 74/14).

blasts (figs. 9, 10); the center is occupied preferably by unorderly disposed collagen fibres or sclerotic tissue. This structural pattern may be said to be the general one; but great individual variations may be found which offer special interest. The fibroblasts always have definite boundaries, they may be separated each one from the other, even in the very periphery, by collagenous fibres. The fibrous tissue may be loose and seemingly also edematous. Bundles of smooth muscle fibres may be present in some areas of the central portion or other parts of a tumor (fig. 11A and B). In other cases the quantity of smooth muscle tissue may become very considerable (figs. 11C, 16, 17).



FIG. 12. Proliferation of cells lining vascular spaces. 53 inj. of 40 µg of estrad. (benz.). Small blood vessel in a uterine fibroid. $\times 240$. (fem. G.p. I.19).

Different details of structure shall be discussed in the following three paragraphs.

1. Proliferation of cells of the mesenchym

We have referred to the cells which compose the thick peripheral belt of the tumor as fibroblasts. But this comparison is only approximate. There are considerable variations in the size of the cell and in the shape of the nucleus.

A remarkable feature is the enlargement of the endothelial cells of the serosa on the tumor itself or nearby. Sometimes clusters of these enlarged cells may be found (fig. 10B). But in general, the peripheral zone of fibro-

blasts is covered with flattened cells (fig. 9B, 10A) beneath which the thick belt of fibroblasts is located. At first glance there would seem to be no doubt of there being a proliferation of the endothelial cells of the serosa; but it would be difficult to say whether the fibroblasts of the peripheral belt are really related to these endothelial cells. It seems more probable that the fibroblasts originate fundamentally from the proliferation of subendothelial



FIG. 13 A

cells. The endothelial or subendothelial elements of the serosa are certainly not the only ones which enter into atypical proliferation when subject to the prolonged action of estrogen. With a sufficient number of observations one comes to the definite conclusion that there is, under the influence of the prolonged action of estrogens, an atypical proliferation of different types of the cells of the mesenchym. Various examples may be given.

One may find pictures which suggest that proliferation of cells related to the wall of vascular spaces may partake, or have even an essential part, in

the origin of an estrogen-induced nodule. This was, for instance, the case with small intramural uterine nodules which, indeed, must be a very rare phenomenon as they were found only in one case in clefts of the muscular septum between the two uterine horns. The clefts, probably lymphatic spaces, were lined with endothelium. But here again, as with the peritoneal

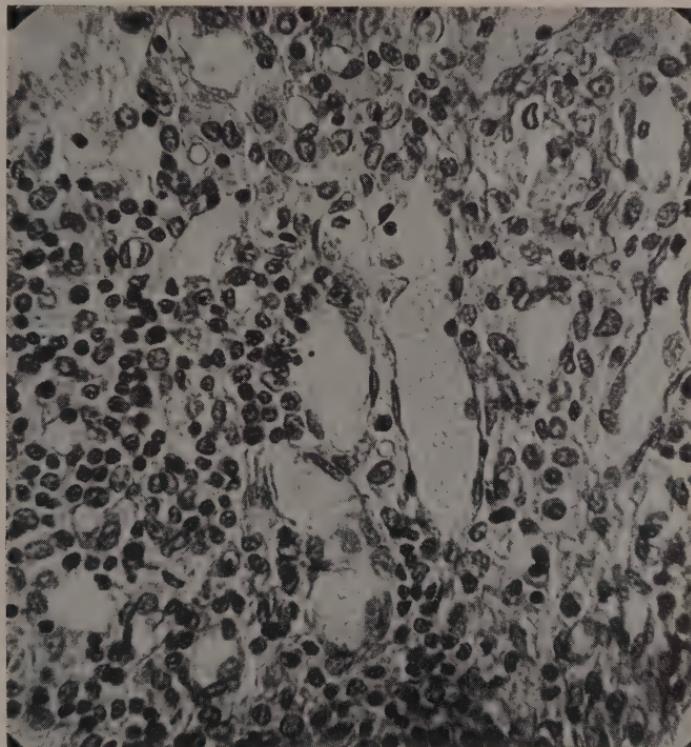


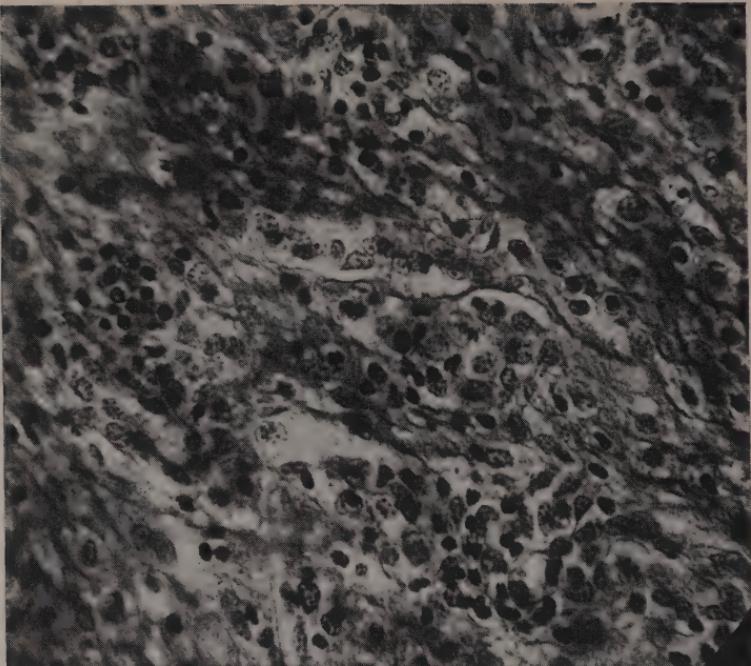
FIG. 13B

FIG. 13. Proliferation of cells lining vascular or lymphatic spaces in a large apical tumor. 40 inj. of 80 µg of estrad. (diprop.), 90 days. (fem. G.p. VI.38). A. Cluster of cells in the center of the tumor; the cluster is surrounded by collagen fibers. $\times 600$. B. Great abundance of cells in immediate contact with vascular spaces, in the vicinity of adipous tissue. The cells are of different types. $\times 400$. The cells both in A and B probably due to proliferation of mesenchyme around the spaces.

serosa, it is most likely that the proliferating cells originated not from the endothelium lining the space but from the underlying mesenchymal cells, though the endothelium of capillaries may be found thickened. Other examples of proliferation of cells related to the wall of vascular or lymphatic spaces are given in figs. 12 and 13.

Remarkable is also the abundance of cells rich in cytoplasm and of dif-

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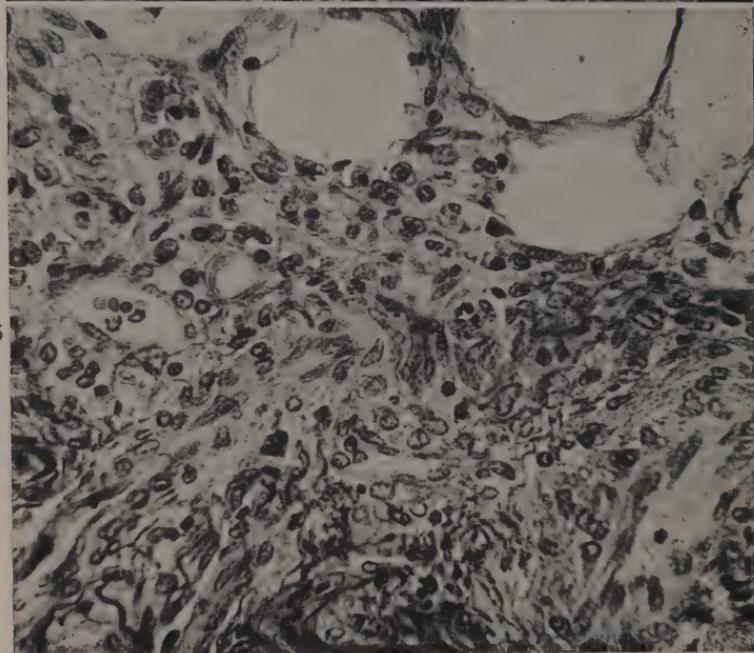


FIG. 14. Tumor between spleen and stomach. 33 inj. of 80 µg of estrad. (benz.).
81 days. Abundance of various types of cells. $\times 400$. (fem. G.p. II.38).

FIG. 15. Proliferation of cells on the border between fibroid and adipous tissue.
45 inj. of 80 µg of estrad. (17-benz.-3-n-butyryr.), 104 days. (male G.p. VIII.15).

ferent nuclear types which may be found within the adipous tissue or in contact with it (figs. 13B, 14, 15). I am unable to establish whether these

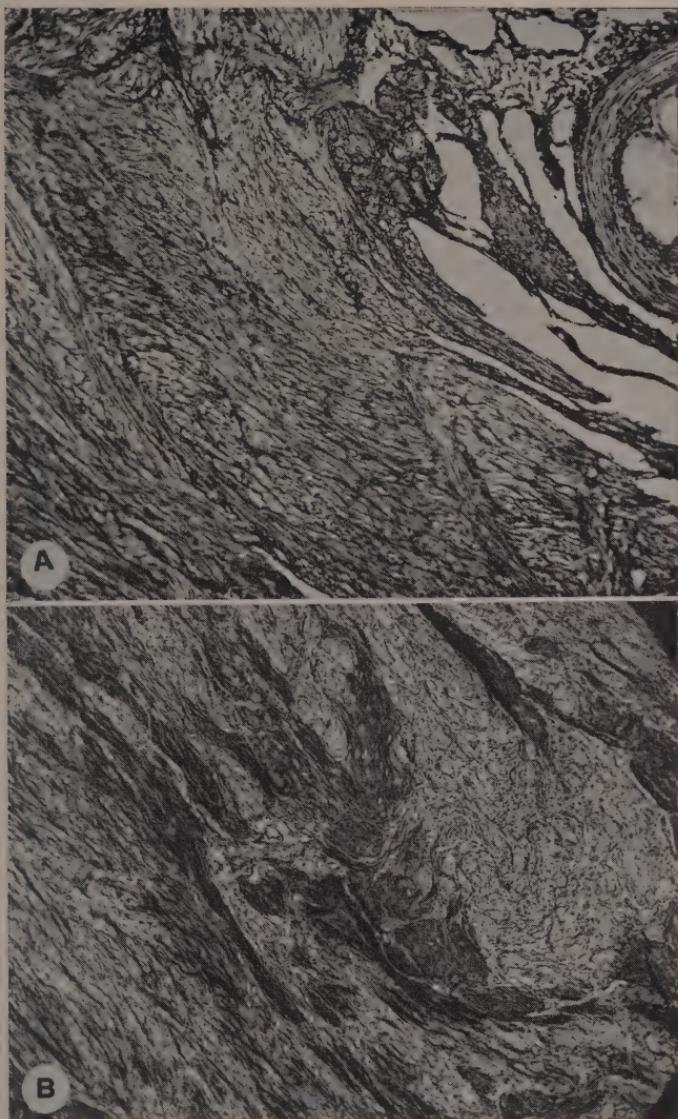


FIG. 16A AND B

cells are undifferentiated cells of the adipous tissue, or whether they are related by origin to the cells of vascular spaces. There is indeed in these clusters of cells a wealth of capillaries (see especially figs. 13B, 14).

A most valuable description of the histogenetic aspects of these tumors has been given recently by Mosinger (1946) who lays special stress on the

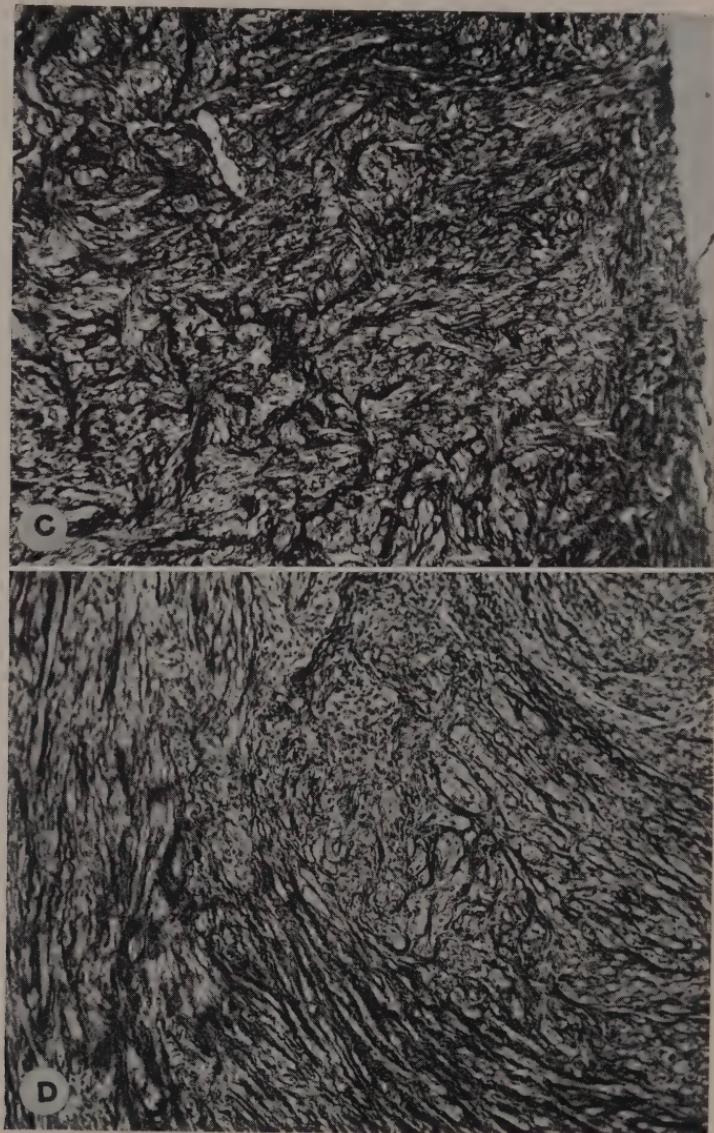


FIG. 16C AND D

proliferation both of endothelia and of perivascular histocytes which are supposed to transform subsequently into fibroblasts and muscle fibres (Mosinger, 1946, pp. 110, 120, 140). Mosinger (1947) agrees that fibroids and

fibromyomata are indeed the most frequent types of estrogen-induced tumors; but he emphasizes that other tumors deserve the name of lymphocytoma and angioma.

2. Fibroid, fibromyoma and myoma

Different structural aspects have been mentioned which show that there was in the estrogen-induced nodules, due to the proliferation of the serosa or subserosa, a strongly pronounced tendency to production of collagenous fibres: the fibroblasts of the peripheral belt may be surrounded by thick collagenous fibres and the nearer to the center the greater is the space



FIG. 16E

FIG. 16. Estrogen-induced proliferation of smooth muscle tissue. A. Myomatous patch of the mesosalpinx. 32 inj. of 80 µg of estrad. (benz.) in female G.p., 80 days. The tube is seen to the right. Wolffian tubules also may be included in the myoma. $\times 90$. (G.p. II.21). B. Large apical fibromyoma. $\times 45$. Same anim. as figs. 12 and 19A (I.19). C. Subserous uterine myoma (saddle tumor). Subcut. pellet containing but 5 per cent of estrad. 244 days. $\times 90$. (G.p. CXVIII.5, see the tumor in fig. 34.) D. Fibromyoma of the hilus of the spleen in male. Same tumor as fig. 11A. This part consists mostly of smooth muscle fibers. $\times 90$. (G.p. VIII.1). E. Estrogen-induced subserous circular muscle layer. Subcut. pellet containing but 3 per cent of estrad., 245 days. $\times 45$. (G.p. CXVIII.39).

occupied by connective tissue. This tendency already becomes manifest when the nodule is still very small; production of collagenous fibres accompanies so to say, or partakes in, the very process of building and growing of the nodule *from the very beginning*. Later on sclerotic and probably also hyalinized tissue may prevail. But amidst this tissue fibroblasts rich in cytoplasm may still be found.

Presence of bundles of smooth muscle fibres in the more central part of these tumors has also been mentioned above. Smooth muscle tissue is sometimes more abundant in uterine tumors. Tumors of the mesosalpinx, or the "apical" tumors (figs. 3, 8) have to be mentioned here in the first place.

Whereas often the admixture of smooth muscle tissue is less conspicuous, there are other cases in which myomatous masses may be found (fig. 16A). They may be due to engorged hyperplastic patches of this tissue present in the normal animal's mesosalpinx near the tube. The tube itself and Wolffian tubules may be found near or amidst the myomatous masses, or they may be found amidst the smooth muscle tissue. An abundant admixture of smooth muscle tissue may be found also in subserous uterine tumors where the ample myomatous patches may be due to the proliferating tumoral tissue displacing and engorging subjacent parts of the myometrium.

Whereas we must admit that in these apical and subserous fibromyomata preexistent, though hyperplastic, smooth muscle tissue may become incorporated into the estrogen-induced tumor, there is full evidence that neof ormation of smooth muscle fibers also takes place under the prolonged estrogenic stimulus. Indeed, we were often unable to establish clearly whether the proliferated large spindle shaped cells were still fibroblasts or smooth muscle fibres, or *transitory* between both. But unmistakable smooth muscle fibres were also found in the thickened uterine subserosa; here there was no question of their preexistence. The amount of the newly formed smooth muscle tissue may become so considerable that the thickened serosa is transformed into a third myometrial layer (fig. 16E). This layer is circular and it may even surpass in thickness the hyperplastic longitudinal and circular layers normally present in the myometrium.

There were in our work also true uterine fibromyomata or myomata (fig. 16B). We found conspicuous myomatous growth in experiments in which the quantities of estrogen administered in the course of eight months were small; they were absorbed from subcutaneous pellets containing but 3 to 5 per cent of the estrogen as in fig. 16C (Tenorio, 1947). The statement that atypical proliferation of mesenchymatous and epithelial cells was to be elicited with minute quantities of the estrogen was, from the beginning, one of the most striking aspects of our experimental work. The quantity of estradiol absorbed from 3 or 5 per cent pellets was certainly less than 2.7 to 4.5 μg per day (see also p. 43 and 44). Since the myomatous character of uterine tumors was very pronounced in several of these experiments the question arises whether the small quantity of estrogen, and not the large one, is especially favorable to myomatous proliferation. No satisfactory answer can be given as to this; the above-mentioned findings of myomatous patches in apical tumors were made in animals receiving large quantities of estrogen by injection (see fig. 16A).

Another highly interesting statement refers to the finding of myomata in the *male*. These myomata can be seen with frequency in males in which a ligature has been made on the ductus deferens and to which estrogens were administered for several months (Koref et al., 1939; Jedlicky et al., 1939) (fig. 17; fig. 44). There is still another tumoral localization typical of the

male with a preponderance of smooth muscle tissue—the utricular fibro-myepithelioma (p. 67).

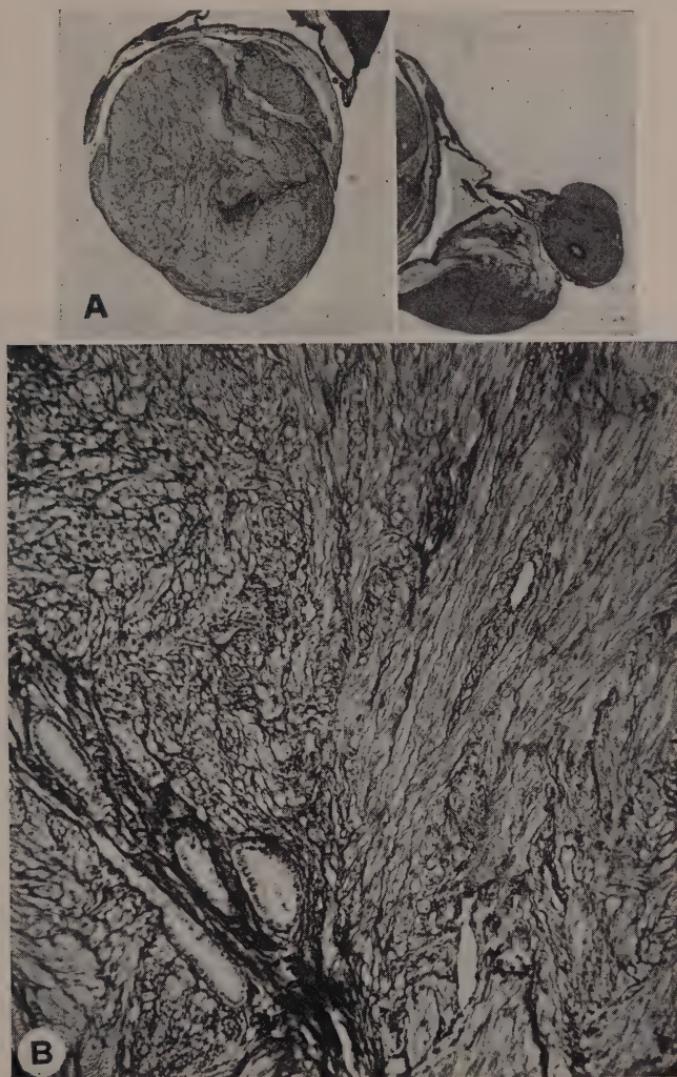


FIG. 17. Inguinal tumor in male guinea pig. 45 inj. of 80 μ g of estrad. (capryl.), 105 days. A. Right—deferent duct with adipous tissue and small fibrous proliferation. Left—tumor. $\times 5$. B. Part of the tumor. Almost pure myoma. The tubules are possibly Wolffian. $\times 90$. (VIII.17).

Highly remarkable was also another statement: a greater wealth of smooth muscle fibres was found in the male even in certain tumoral localizations comparable in, or common to, both sexes. Mention may be made here,

in the first place, of the fibrous reaction in the submucosa. In the uterus and vagina the fibrous patches of the submucose, or even a polypous formation (fig. 18), were composed entirely, or almost entirely, of collagen fibres; on the contrary, a fibromyomatous growth was found in the submucosa of the seminal vesicles in a male to which estrogen was administered during more than two years. Considerable quantities of bundles of smooth muscle fibres were present, in males, in fibroids of other localizations as between spleen and stomach (fig. 16D) or on the kidney. It seems paradoxical that myomatous proliferation, a tumoral structure so typical of the female, should be found in the male, whereas in the female tumors prevailed in which, in general, there were only small quantities of the smooth muscle tissue. There is

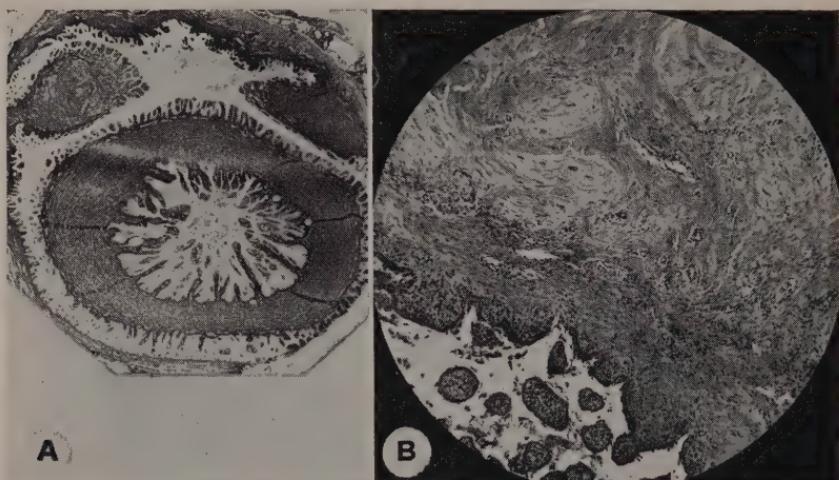


FIG. 18. Tumors of vaginal submucosa. 129 inj. of 20 µg of estrad. (benz.), 310 days. A. In the center the uterine cervix. Two pedunculated fibroids. $\times 5$. B. The tumor consists of dense collagen tissue with very scarce cells. $\times 45$. (G.p. I.22).

still another remarkable difference between the two sexes: the incidence of estrogen-induced abdominal fibroids is, in the male guinea pig, much smaller than in the female (ch. 5).

There were in our work all transitions between tumors predominantly fibrous and predominantly myomatous. The quantity of smooth muscle fibres being so variable and often very limited we came to the conclusion that "fibroid" may be a more appropriate name for these experimental tumors than "fibromyoma". All the more so, as in human pathology one has become accustomed to call "fibroid" uterine tumors of a very variable content of fibrous and myomatous tissues.¹

¹ In the Editorial of the *Lancet* (1946) dedicated to the lectures I delivered in the Royal College of Surgeons, the following statement has been made with reference

Although estrogen-induced experimental fibroids cannot be structurally identified with uterine fibroids in women, our findings suggest that the latter are related to an endocrine disturbance somewhat similar to that produced in our experimental work. But one must not oversimplify the problem, since in human pathology fibroids similar in all the structural details to those induced experimentally by estrogen are apparently rare. One of these details shall be discussed in the following paragraph.

3. Invasive faculty of the experimental fibroid

The most relevant difference both in structure and in behaviour of uterine fibromyoma in women and estrogen-induced fibroids in the guinea-pig is the invasive faculty of the latter (Lipschutz, Vargas and Iglesias, 1938; Lipschutz and Vargas, 1941; Lipschutz, 1942b).

The estrogen-induced tumor may invade the smooth muscle cover of the *intestine* (fig. 19A), or the muscular coat of the urinary bladder. But the tumor never reached the mucosa. As to the *uterus* the limit between tumor and myometrium may be a sharp one (fig. 9A) especially when the tumor, subserous or parametrial, is still small. Later on the limit may become less distinctive (fig. 23A and B); bundles of muscle fibres may be engorged by the tumor. Even more impressive was the invasion of striated muscle by fibroids of the *diaphragm* or the *abdominal wall* (fig. 19B), and of the *pancreas* (fig. 20), and though very rarely, of the liver. Disintergration of striated muscle tissue and especially of the pancreas (fig. 20B) and even destruction of glandular tissue (fig. 20C) was sometimes very striking. It was remarkable that also fibromyoma was invasive (fig. 20D).

Fibroids in close contact with organs other than those mentioned above did never invade: tumors on the surface of the spleen (fig. 21) or of the kidney (fig. 22) were separated from the organ by a sharp line. With the uterus invasion was seemingly more superficial and not destructive (fig. 23).

Full corroboration of our statements on invasive growth of fibroids was given both by Von Wattenwyl (1944) and Mosinger (1946).

Invasive fibroids of the abdominal wall have been reported also in human pathology (Mosinger, 1946, pp. 275, 306); they can become sarcomatous and produce metastases. Mosinger (1946, p. 24) labels the estrogen-induced fibroid "fibrosarcoma." According to our feeling it is preferable to avoid this designation as these experimental tumors have no autonomous growth, do not produce metastases, and do not grow when transplanted.

to our using formerly the term "fibromyoma" and now "fibroid": "It appears now, however, that the localized fibrous hyperplasia of guinea pigs do not contain any plain muscle fibers; they are composed of fibroblasts and collagen. . . ." This is a misunderstanding of which as I admit I myself may have been responsible not making things sufficiently clear in my lectures or in the manuscript of the lectures.

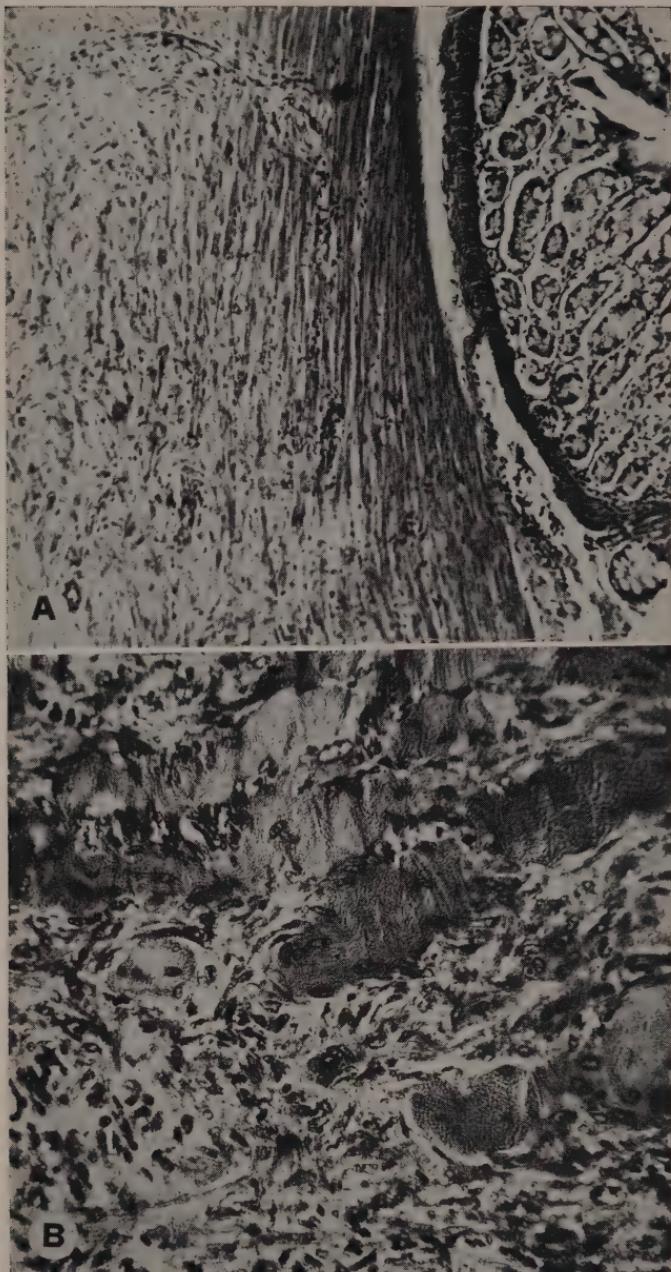


FIG. 19. Invasion of muscle tissues. A. Invasion of muscular coat of the rectum by a pelvic tumor. (I.19; same animal as fig. 12 a. 16B.) $\times 90$. B. Invasion and destruction of striated muscle. Tumor of abdominal wall. (I.12; same animal as fig. 6B).

The structural differences between experimental fibroids in guinea pigs and uterine fibroids in women are not due to a differential behavior of the

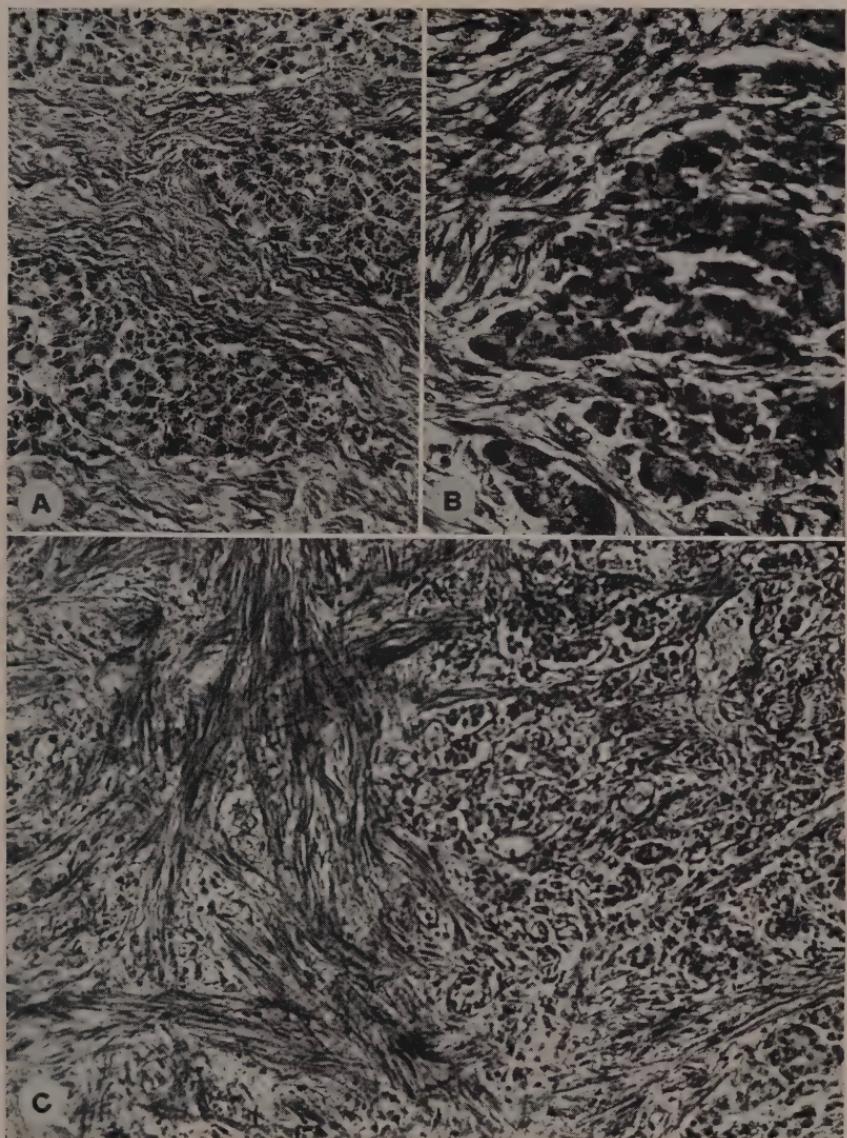


FIG. 20A, B AND C

zoological species. It is significant that the experimental fibroids were structurally different also from the only spontaneous uterine fibroid I was able

to find in the guinea pig (fig. 24A). The spontaneous parametrial tumor was a true fibromyoma very similar to those in women; the bundles of muscle fibres were orderly disposed (fig. 24B). In another untreated animal a small nodule of hyperplastic muscle fibres was found in the uterine wall (fig. 25).

C. "TUMORAL SEED" AND "DISSEMINATED FIBROSIS"

The size of the experimental fibroids varies greatly—they may be just visible to the naked eye, or they may attain a diameter of several

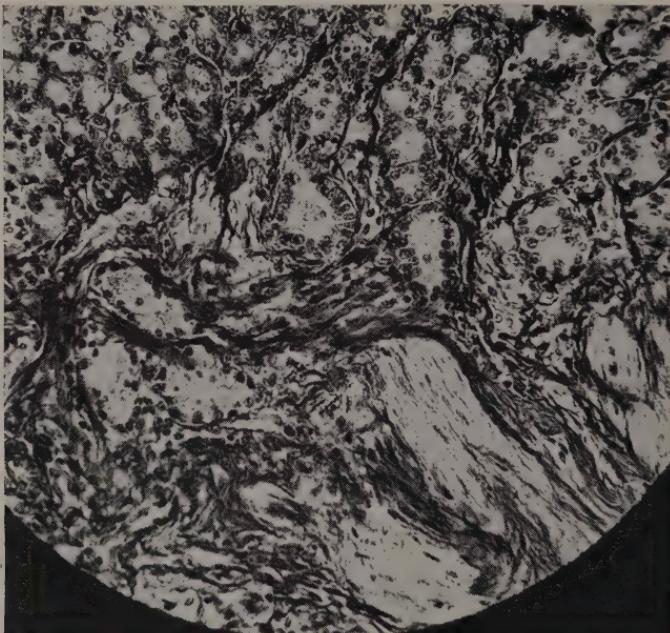


FIG. 20D

FIG. 20. Invasion, disintegration and partial destruction of pancreas. A. Enormous thickening of stroma between pancreatic lobules. 108 inj. of 80 µg of estrad. (benz.), 240 days. $\times 200$. (fem. G.p. I.5). B. Disintegration of lobules. 51 inj. of 40 µg of estrad. (benz.), 121 days. $\times 240$. (fem. G.p. I.3). C. Disintegration and destruction of glandular tissues by fibroid. 49 inj. of 80 µg of estrad. (benz.), 122 days. $\times 125$. (fem. G.p. II.14). D. Invasion of pancreas by fibromyoma. $\times 200$. (Same animal as fig. 11C).

centimeters (fig. 1 to 8). They may appear as small nodules or excrescences already about two to four weeks after the beginning of administration of estrogens (figs. 26, 27).

The small nodules were often present in considerable number especially on the surface of the spleen (fig. 7) and the surrounding regions of the abdominal wall and the stomach (see diagrams, fig. 32 and others) (Lipschutz and Vargas, 1939, 1941a). We shall have to refer to this tumoral seed repeatedly. It is the first sign of a fibrous tumoral reaction, or the most sensi-

tive tumorigenic test; for this reason the tiny nodules attracted considerable interest in the course of our experimental work.

The structure of the tiny nodules is similar to, but seemingly not identical with, that of the large fibroids. As in other fibroids here again spindle shaped

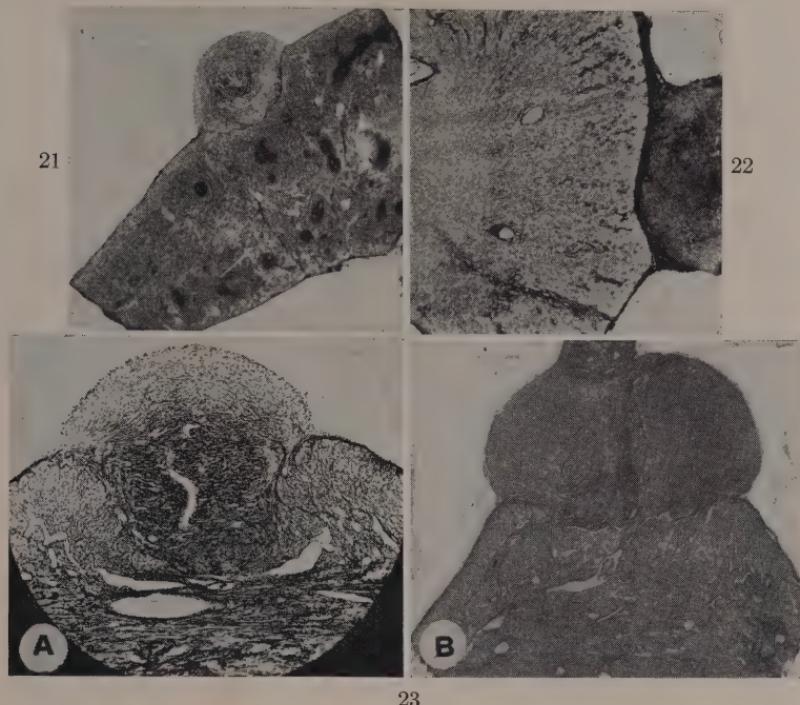


FIG. 21. Sharp limit between subserous tumor and spleen. 38 inj. of 500 µg of stilbestrol, 95 days. $\times 5$. (fem. G.p. II St.10).

FIG. 22. Sharp limit between subserous tumor and kidney. $\times 5$. (male G.p. VIII.6; same animal as fig. 11B).

FIG. 23. Relation between uterine tumors and the myometrium. A. Parametrial tumor of uterus. 45 inj. of 250 µg of stilbestrol, 108 days. $\times 23$. (IISt.5). B. Parametrial tumor of uterus. 42 inj. of 400 µg of estrone, 95 days. (V.28). $\times 5$ (erroneously given as 123d. with estrad. in Canc. Res. 1:236, fig. 9). The limit between uterus and tumor is in A and B a sharp one on the border. But in the center the tumor presses against the longitudinal muscular layers and they have almost disappeared in B.

cells may be found in the periphery and sclerotic tissue in the center. But these tiny nodules seem to have a stronger tendency to sclerotization; they consist sometimes of collagen fibres only. In other cases cells rich in protoplasm predominate (fig. 27).

Another feature of peritoneal reaction in the guinea pig under the influence of a prolonged action of estrogen must be mentioned here—the

fibrous strands of the mesentery, of the abdominal wall and of the hilum of the spleen, a condition which may be called "disseminated fibrosis." There

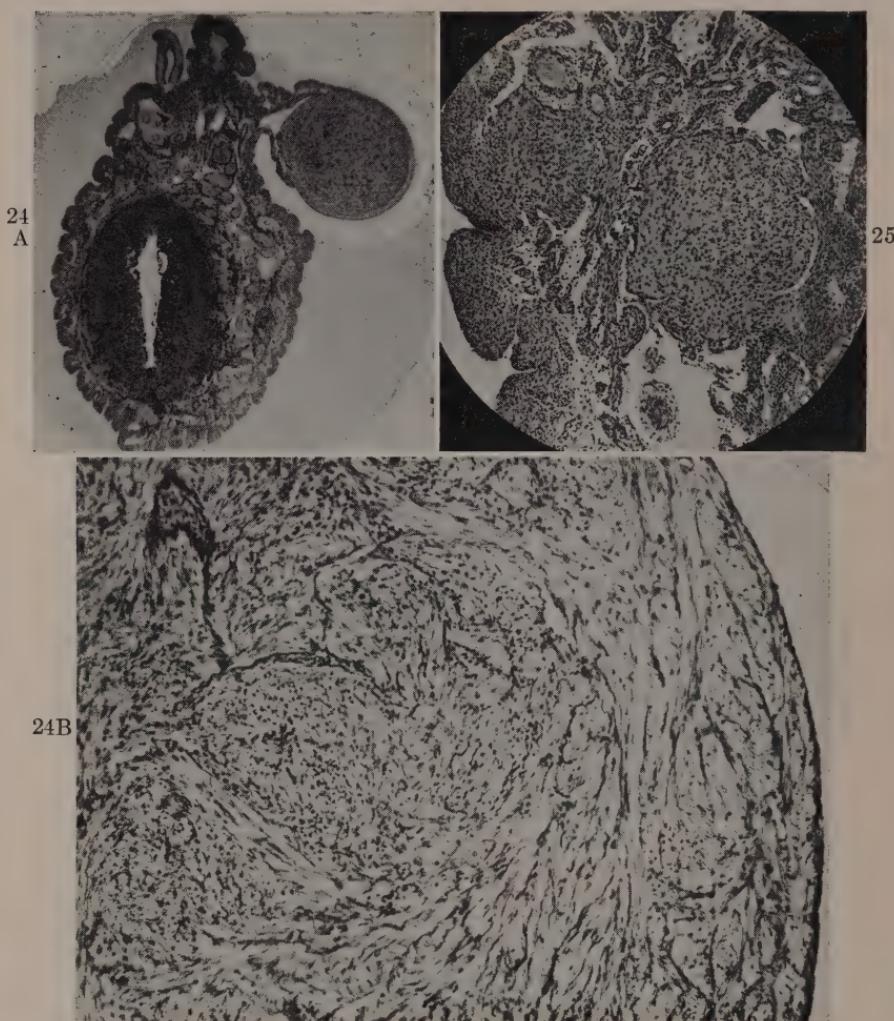


FIG. 24. Spontaneous fibromyoma in the guinea pig (C.39). Untreated animal. A. Normal aspect of uterus with tumor of the parametrium. $\times 10$. B. The tumor at $\times 90$.

FIG. 25. Small intramural nodule in the uterus of an untreated guinea pig. The nodule consists of large cells with clear protoplasm probably hyperplastic muscle fibers. $\times 45$. (C.40).

is sometimes a thickening of the whole mesentery and considerable retraction (Iglesias 1938). With smaller doses these fibrous strands are the only

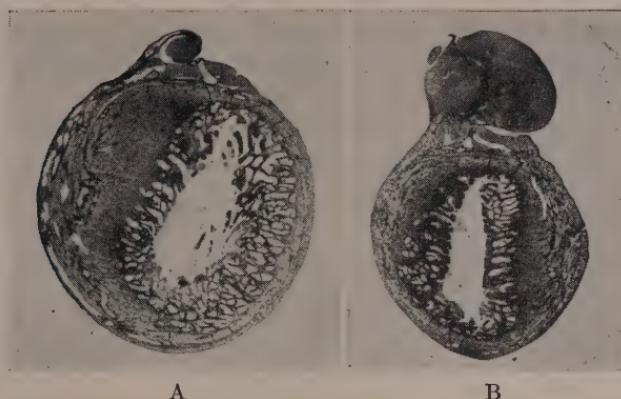


FIG. 26. Early appearance of uterine parametrial fibroids when fibromatogenic conditions are most favorable: animals with subcutan. pellet of estradiol. A. 31 days. $\times 5$. (II, tabl. 13). B. 49 days. $\times 5$. (II, tabl. 17).



FIG. 27. Early appearance of subserous nodules on the surface of the spleen under fibromatogenic conditions most favorable. A. 7 inj. of 10 μ g of estrad. (17-capryl), 15 days. $\times 45$. (XII.33). B. Estrad. from subcut. pellet, 22 days. $\times 200$. (II, tabl. 3).

manifestation of a peritoneal reaction (Vargas 1942) though in general accompanied by the tumoral seed on the spleen and the surrounding parts. In the male this is the rule even with large doses (Koref et al., 1939). It seems that these fibrous strands develop more readily in older females; but we are not quite sure of this implication of age (work with Vargas and A. Zepeda). It is known from the work of different authorities that estrogen induces proliferation of conjunctive tissue in many parts of the body (see ch. 5); our statements with estrogen-induced abdominal fibroids show that

TABLE 1

*List of estrogens which have been used to induce abdominal fibroids**Free natural estrogens and their derivatives:*

- (1) α -estradiol
- (2) estrone
- (3) estriol
- (4) equilenin
- (5) α -dihydroequilenin (Kamm)
- (6) β -dihydroequilenin (Kamm)
- (7) β -estradiol

Esters of α -estradiol:

- (8) 3-benzoic
- (9) 3-17-propionic
- (10) 17-caprylic (Miescher)
- (11) 3-n-butyric-17-benzoic (Miescher)

Artificial estrogens and their esters:

- (12) diethylstilbestrol (Dodds et al.)
- (13) propionic ester of diethylstilbestrol
- (14) hexestrol (Dodds et al.)
- (15) benzestrol (Blanchard et al.)
- (16) propionic ester of benzestrol
- (17) caprylic ester of benzestrol
- (18) bisdehydrodoisynolic acid (Miescher)
- (19) 3-methyl-ether of bisdehydrodoisynolic acid (Miescher)
- (20) methyl ester of 3-methyl-bisdehydrodoisynolic acid (Miescher)

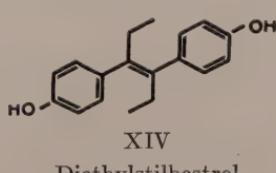
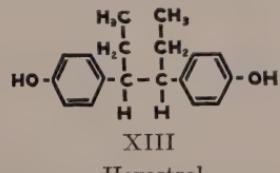
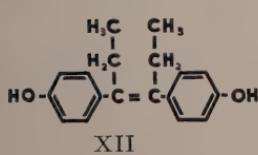
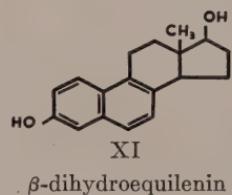
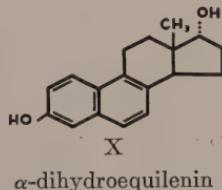
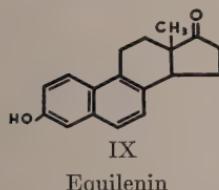
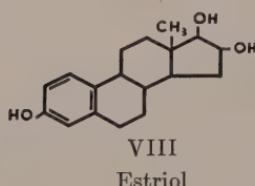
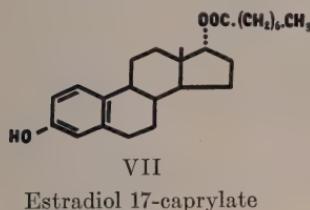
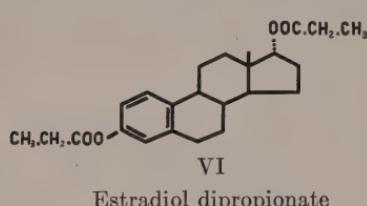
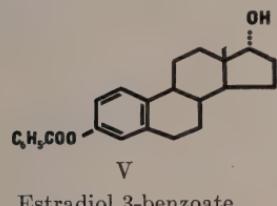
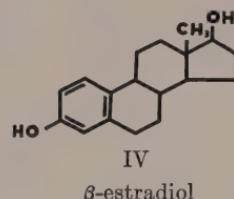
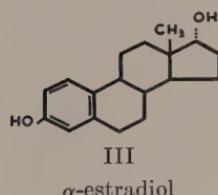
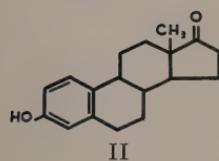
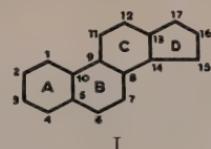
these localized tumoral growths are but a local manifestation of an experimentally established general disease of conjunctive tissue.

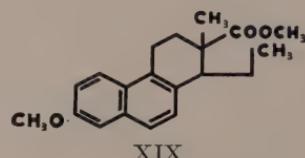
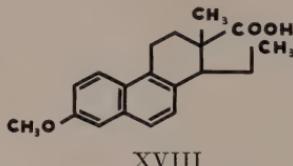
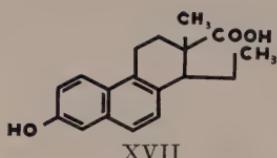
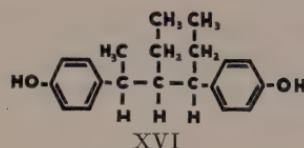
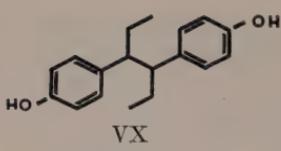
D. ESTROGENS USED

Our first experiments were made with the 3-benzoic ester of α -estradiol (Lipschutz and Iglesias, 1938); this ester was at that time preferably used by the practitioner. Subsequently we tried other estrogens as shown in table 1.

We were able to elicit fibroids with all the estrogenic compounds tried.

Our results give evidence that the fibromatogenic faculty of the estrogen is not dependent on any special structure of the estrogenic compound. In





other words: fibromatogenic action is, under certain experimental conditions, concomitant with estrogenic activity.

There were indeed very considerable differences as to the fibromatogenic activity of the compounds tried. Reference to these differences will be made in the following pages, as also to the relevance of these differences from the point of view of the dynamics of tumorigenesis in general. In certain cases, as with the comparative action of ovarian (1 and 2) and urinary (3 to 6) estrogens, the difference in fibromatogenic activity is certainly only a manifestation of their different estrogenic activity: under equal timing conditions of administration—absorption from subcutaneously implanted pellets—urinary estrogens which are less estrogenic, are also many times less fibromatogenic than ovarian estrogens. It is the same with β -estradiol whose estrogenic activity is considerably smaller than that of α -estradiol; using the same method of administration fibroids were produced only exceptionally and only with quantities many times those of α -estradiol (Segaloff and Iglesias, unpubl. work).

On the contrary, the comparative fibromatogenic activity of free and esterified hormones has nothing to do with differences in their estrogenic activity. The most striking example is offered by the 17-caprylic ester of estradiol. Its estrogenic activity in the rat is only half that of the free hormone (Miescher et al., 1938); but the fibromatogenic activity of this ester when given thrice weekly by injection is fifty to hundred times greater than the fibromatogenic activity of free α -estradiol. This is due to the protracted action of the ester as shown by the following: when these compounds—the free hormone and the 17-caprylate—are administered through absorption from a subcutaneously implanted pellet then there is no difference any more between them as to their fibromatogenic activity.² Greater fibroma-

² Even the difference between the estrous-inducing total dose of say estradiol and estriol can be reduced from 1:280 when given in 2 injections, to 1:7 when given in 4 injections (Emmens, 1939; see table 8).

togenic activity of the stilbene derivatives compared to that of estradiol in injection experiments also can be explained as due to more protracted action. These questions are very relevant in our context; they shall be discussed more fully in ch. 3.

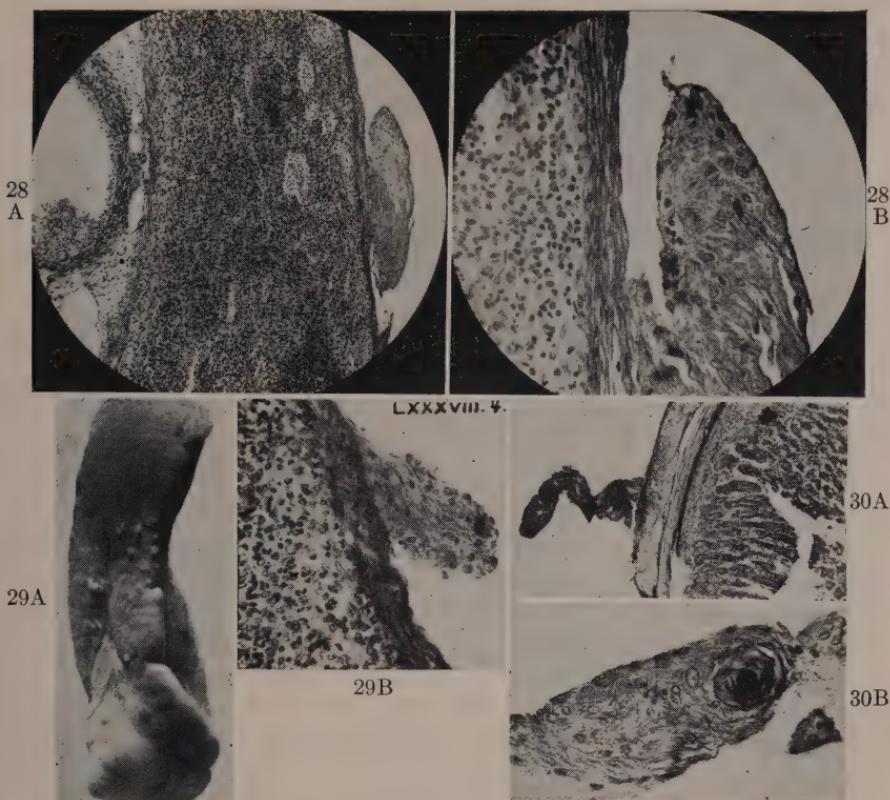


FIG. 28. Fibrous nodules on the surface of the spleen induced by intrasplenic auto-plastic ovarian graft. 62 days after transplantation. A. The nodule is situated above the graft. $\times 45$. B. Part of the nodule at $\times 200$. (LII.60).

FIG. 29. Same, 203 days after transplantation. A. Greatly increased ovary on the surface of the spleen. Numerous nodules at a certain distance of the graft. $\times 2$. (LXXXVIII.4). B. One of the smaller nodules at $\times 200$.

FIG. 30. Fibrous nodules on the surface of the stomach in an animal with an auto-plastic ovarian graft. 62 days after transplantation. (LXXXVIII.44). A. $\times 45$. B. $\times 200$.

E. EXPERIMENTAL FIBROUS NODULES INDUCED WITH THE ANIMAL'S OWN ESTROGENS

Mention has been made above of the tumoral seed on the surface of the spleen which appears so frequently when one is working with estrogen-induced fibroids in the guinea pig. A similar tumoral seed may appear under

certain experimental conditions in animals not injected with hormones. An ovary is grafted into the spleen of the guinea pig; the ovarian autograft takes and develops splendidly in most of the cases, especially when the second ovary has been removed. Two to five months later small nodules may appear on the surface of the spleen (figs. 28 and 29). These nodules were structurally identical with those induced by the administration of estrogens (fig. 27). Their appearance may be explained by the local action of estrogen continuously circulating in the spleen before being drained into, and inactivated by, the liver (fig. 85). The nodules appeared, in experiments with intrasplenic grafts, sometimes, though very rarely, also in the surrounding regions as the stomach (fig. 30) and abdominal wall. It would be difficult to explain this special localization.

The related phenomenon is a very striking example of how the animal's own estrogens may become able to elicit tumoral growth. Here the fundamental question arises about the conditions by which the estrogen which is a physiological morphogenic substance with a selective action on certain tissues, becomes a fibromatogenic or tumorigenic agent, i.e. acquires a faculty so contrary to the physiological sense of the hormone.

Chapter 3.

FIBROMATOGENIC VERSUS PHYSIOLOGICAL ACTION OF ESTROGENS

A. THE FIBROMATOGENIC TIMING CONDITIONS

1. The fibromatogenic threshold concentration and the continuous maintenance of the latter

The first insight into the dynamics of the break from a physiological to a fibromatogenic action, or from a morphogenic to a tumorigenic action of estrogens, was obtained when we tried to produce abdominal fibroids with free α -estradiol instead of the benzoic ester as used in our work at the beginning.

Uterine and other abdominal fibroids were elicited when the equivalent of 10 μg (microgram = 0.001 milligram) of α -estradiol administered in the form of the benzoic or dipropionic ester were injected thrice weekly during three months. In some cases small fibrous nodules, especially on the spleen, may be induced already with half of this quantity. On the contrary, no less than 200 to 400 μg of free α -estradiol or estrone have to be injected thrice weekly to induce abdominal fibroids (Bellolio, 1939; Rodríguez, 1940; Lipschutz, Rodríguez and Vargas, 1939; summaries Lipschutz, 1942a, b, c). In other words: under the given experimental conditions the 3-benzoic ester of α -estradiol was many times more fibromatogenic than the free hormone. How could this difference be explained?

I suggested that the fibromatogenic action may depend upon the continuous maintenance of a threshold concentration of estrogen in the blood. This suggestion was based on the following considerations.

One of the most striking differences between the free hormone and its esters is known to be the smaller rate of absorption of the latter. One must then suppose that a continuous maintenance of a threshold concentration will be more easily effected with the esterified hormone than with the free one. The free hormone when subcutaneously injected and rapidly absorbed is known to be also as rapidly inactivated (fig. 31A). When, say, three injections of 200 μg per week are made, the concentration of estrogen in the blood will descend in the interval between two injections, below the supposed fibromatogenic threshold. This threshold will not be main-

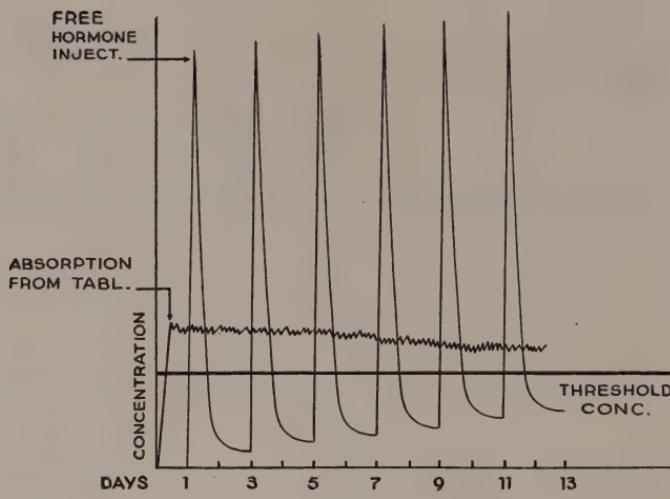


FIG. 31A

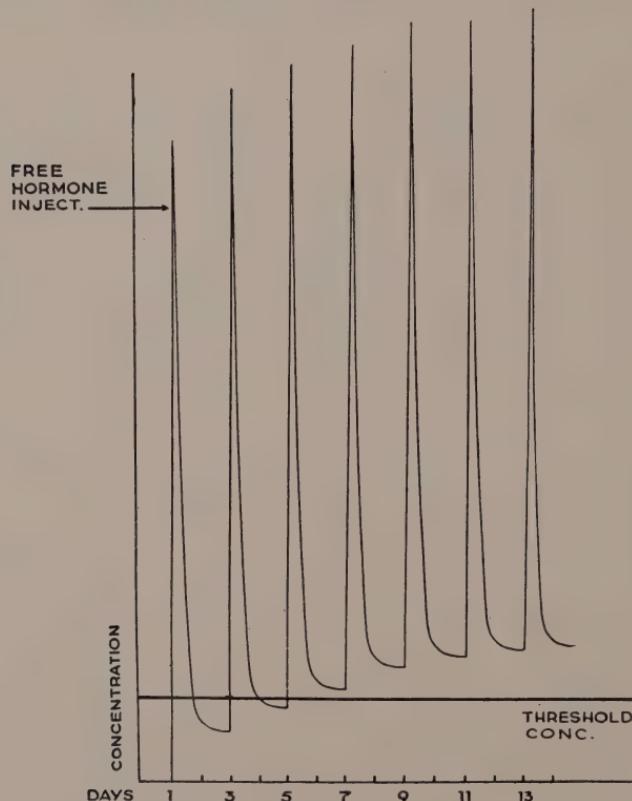


FIG. 31B

tained unless very considerable quantities, say 400 μg , are injected thrice weekly, i.e. quantities so great that their absorption or inactivation—the latter is the more probable—is not completed in the interval between two injections (fig. 31B). Quite different is the situation as to absorption and inactivation with the esterified hormone having a slow absorption rate (fig. 31C). If a sufficient quantity, say 5 to 10 μg , is injected, absorption will not be completed in the interval between two injections; when three injections of the ester are given per week there will be a continuous flow of estrogen from the site of injection towards the body fluids. In other words:

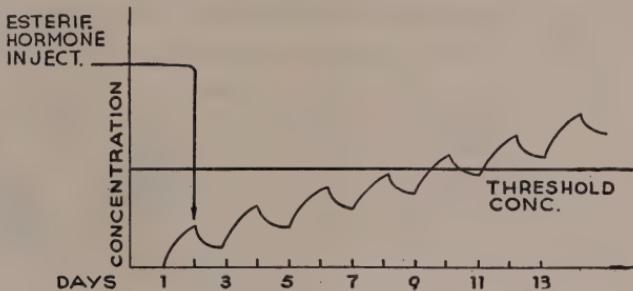


FIG. 31C

FIG. 31. A. When 200 μg of the free hormone are injected the concentration in the blood increases rapidly and considerably on account of rapid absorption. But there is also rapid inactivation, and before the next injection is made two days later the concentration may diminish beneath the threshold. On the contrary, with the continuous absorption from a subcutan. pellet maintenance of the threshold concentration is obtained with daily quantities much smaller than through injections. B. With the injection of considerable quantities of the free hormone—400 μg thrice weekly—the threshold concentration may be maintained because inactivation is not completed in the lapse between two injections. C. Absorption of the esterified estrogen is a slow one and so is probably also inactivation. Even when small quantities of the esterified hormone are injected every second day absorption and inactivation is not completed between two injections. Thanks to this the threshold concentration can be maintained with small quantities. For further explanation see text.

slow absorption will allow for a continuous maintenance of a certain threshold concentration with quantities of estrogen much smaller than those of the free hormone.

2. Three different ways of experimental approach and evidence

There was no possibility of verifying our suggestion about the bearing of a continuous estrogenic threshold concentration on the fibromatogenic action of estrogens by direct determination of the hormone in the blood of our small experimental animals. But proof in favor of our suggestion was given by demonstrating that the estrogen acquires a greater fibromatogenic faculty the nearer the mode of supply allows for continuous absorption.

a. Fibromatogenic activity of minute quantities of the 17-caprylic ester of estradiol, and of artificial estrogens. Miescher's 17-caprylic ester of estradiol has been shown to have a more prolonged action than the benzoic and dipropionic ester (Miescher, Scholz and Tschopp, 1938). When for instance 250 µg of the 17-caprylic ester are injected into a castrated rat estrous lasts 28 to 42 days, instead of 5 to 10 days with the free hormone, or 10 to 19 days with the dipropionic ester (Mello and Franke 1945). Will the 17-caprylic ester be more fibromatogenic than the benzoic or dipropionic one? Comparative experiments with the three esters mentioned were obtained in a group of 70 animals (Lipschutz, Vargas, Baeza and Baeza, 1941). There was

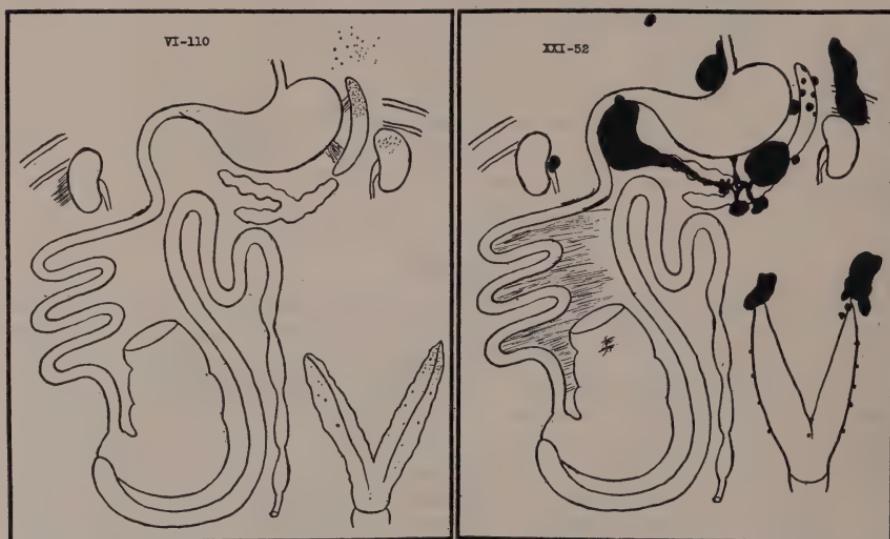


FIG. 32. Comparative fibromatogenic action of two esters of estradiol. 41 inj. of 10 µg of estrad. (diprop.), 92 days. Maximal reaction. Hypertrophy of the ventral ridge of the uterus (VI.110). B. 36 inj. of 5 µg of estrad. (17-capryl.), 84 days (XXI.52).

in almost all animals some conjunctive response as fibrous nodules on the spleen and the surrounding parts, or fibrous strands disseminated in the abdominal cavity. But there was a very pronounced difference between the various groups: quantities of estradiol just sufficient to induce some fibromatogenic reaction, when given in the form of the benzoic or dipropionic ester, elicited a highly pronounced reaction when given under the form of the 17-caprylic ester (fig. 32). In 57 animals receiving the first mentioned esters the average result was, in arbitrary units, F.T.E. = 1. With the same quantity of the 17-caprylic ester the average was, in 13 animals, F.T.E. = 5. (F.T.E. is defined as the *Fibrous Tumoral Effect*).

A few words may be added here as to how these values of F.T.E. are obtained (Lipschutz and Vargas, 1939b, and the definitive form in Lipschutz and Maass,

1944). The tumors are classified with reference to four different regions: uterine tumors (subserous, mesometrial); apical tumors, or tumors of the mesosalpinx; tumors of the digestive tract and the abdominal wall; and tumors of the spleen. The tumors of each region are characterized according to their size as 0.5, 1 (1.5 to 2.5 mm), 2 (3 to 5.5 mm), and 3 (6 mm or more). The sum of the four values is the F.T.E. Fibrous peritoneal strands are characterized by the value 0.5 and exceptionally by 1. The size of the tumors is not measured but roughly estimated. The maximum value is F.T.E. = 12. The method is certainly a very arbitrary and rough one. But the classification gives fairly reliable data, as has been demonstrated by numerous comparative tests in the course of the years.¹ The greatest error occurs rather with the overestimation of a weak reaction and with an underestimation of a strong one.

Esterification interferes not only with the absorption rate; it protects also against intrahepatic inactivation of the estrogen (see Part III). This possibly contributes to prolong the action of an ester. Thanks to all this fibromatogenic action may be enhanced by certain esterifications in an extraordinary manner: in our experiments with subcutaneous injections the 17-caprylic ester was fifty to hundred times more active than the free hormone (see section c). It is very impressive to find large abdominal fibroids in all animals of a series which have received in the course of three months a total of but 200 µg of estradiol given as the 17-caprylic ester (fig. 32). No less impressive is the fact that tiny nodules may sometimes appear even with quantities much smaller than that (fig. 7), and exceptionally as early as two weeks after having begun administration of the estrogen (fig. 27). Indeed, under these special quantitative conditions the incidence of the tumoral reaction is very small.

Results similar to those with the comparative action of free and esterified estradiol have been obtained in our work with artificial estrogens, free and esterified. Diaethylstilbestrol and hexestrol which are known to be more resistant against intrahepatic inactivation than natural estrogens, are also more fibromatogenic. Small nodules on the spleen, the abdominal wall and the stomach may appear already with 1 to 5 µg of hexestrol injected thrice weekly in the course of three months (Lipschutz, Vargas, Egaña and Bruzzone 1941). Stilbestrol though more fibromatogenic than the natural estrogens is less fibromatogenic than hexestrol. But with the esterification which has been shown by Dodds and others to favor a protracted estrogenic action of stilbestrol goes also a great enhancement of its fibromatogenic activity (Bruzzone, 1942). The same is true for free and esterified benzestrol (Alvear, 1944; Mardini, 1947).

No fibroids have been induced in the guinea pig with the injection of Miescher's bisdehydrodoisynolic acid (XVII; Miescher and Tschopp, private communication; Iglesias, Lipschutz and Mardones, 1950b). This failure to elicit tumors was most probably due to the rapid absorption of this

¹ We shall come back in the Part II to the difficulties and drawbacks of our method of quantitative appreciation.

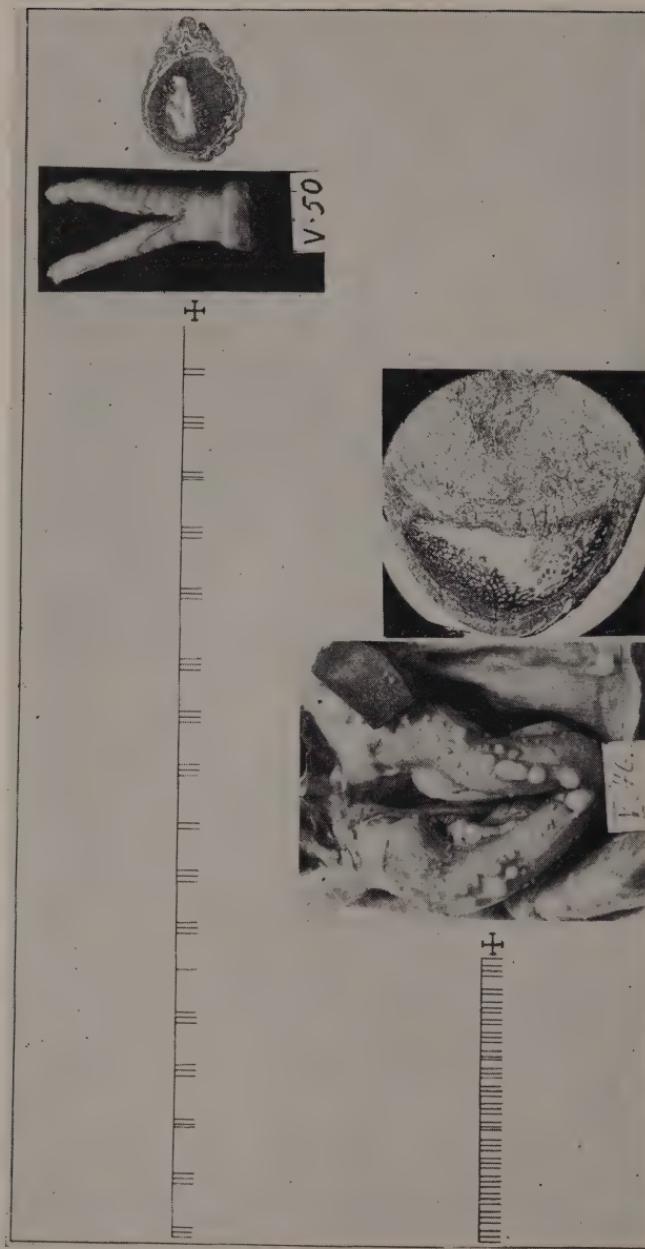


FIG. 33. "Continuous" and "discontinuous" action of estrad. in castrated female guinea pigs. Each mark indicates an injection. Below: Two animals with 47 inj. given in 113 days; great development of the uterus, and large uterine and extrauterine fibroids (V.46: X 0.9; V.44: X 5). Above: Two animals with 47 inj. given in the course of a year; only slight increase of the uterus; no fibroids (V.50: X 1.1; V.49: X 5).

artificial estrogen which as to this behaves similarly to free estrogen when administered by injection (see p. 35 and *c*, fig. 31A). When a continuous flow of bisdehydrodoisynolic acid was established by the subcutaneous implantation of its methylether (XVIII) and of the methylester of the latter (XIX) fibroids were produced (Iglesias, Lipschutz and Mardones, 1950b).

b. Prolonged but discontinuous administration of esters of estradiol is not fibromatogenic. A crucial experiment in favor of our concept has been the following. When a fibromatogenic ester was administered under special timing conditions which did not allow for continuous action there was no fibromatogenic effect at all (fig. 33). Castrated female guinea pigs were given during one week three injections of 20 to 80 μg of the 3-benzoic ester; injections were interrupted for the next two to three weeks till the vagina closed (Lipschutz, Rodríguez and Vargas, 1941). This condition of the genital opening indicates that the body of the castrated female has been freed of most of the remains of the injected estrogen. Then again three injections were given during one week, then interrupted as above for two to three weeks. A total of 47 injections was thus given in the course of 365 days without fibroids being produced.

On the contrary, with the same number of 47 injections given thrice weekly without interruption fibroids were elicited in the course of only 113 days.

These experiments offer also other aspects which are of utmost interest from the point of view of the sexual *rhythm* as an autodifensive device. They shall be discussed more fully in the Part III (ch. 17).

c. Fibromatogenic action of minute quantities of free estrogen administered continuously. As explained above (*a*) abdominal fibroids have been elicited in every female guinea pig by 40 injections of 5 μg of estradiol—or a total of only 200 μg —given as the 17-caprylic ester. On the contrary, 300 to 400 μg of the free hormone per injection—or a total of about 10,000 to 15,000 μg —are necessary to obtain a similar fibromatogenic effect. But things change fundamentally when the free hormone is administered in such a way as to allow for an uninterrupted flow of the estrogen in the body (fig. 31A) as is the case with Deanesly and Parkes' (1938) technic of subcutaneous implantation of pellets. This device has been of the greatest use in our work (Lipschutz and Vargas, 1939a). It was to be supposed from the beginning that absorption of a given steroid will depend on the surface of the pellets. With pellets sufficiently small the quantity absorbed was reduced to 4 or 5 μg per day or to a total of 300 to 400 μg in the course of about three months. Under these experimental conditions an exceedingly small quantity of the free hormone—estradiol or estrone—was shown to be already sufficient to produce uterine and abdominal fibroids though the quantity was 30 to 50 times smaller than that necessary to induce fibroids when given by injections of the free hormone (Thibaut, 1941; Lipschutz, Thibaut and Vargas, 1941).

The fibromatogenic quantity of estrogen was diminished even more when we began using pellets containing a mixture of estradiol with cholesterol. The percentage of the specific steroids mixed with cholesterol did not change in the pellet; that is to say, both cholesterol and the specific steroid were absorbed at a similar rate, without selective absorption (Fuenzalida, 1944; Lipschutz, Bruzzone and Fuenzalida, 1944; for stilbestrol and hexestrol



FIG. 34. Enormously enlarged uterus of a guinea pig with a subcut. pellet containing but 5 per cent of estrad. The weight of the uterus was of 47 g. Duration of the experiment 244 days. Subserous saddle tumor. *Nat. size.* (CXVIII.5). Comp. with figs. 1 to 5.

see Shimkin and White, 1941; for other substances Parkes, 1942). There is full evidence that it is the same with estradiol mixed with cholesterol as has been shown in a series of experiments made in collaboration with Riesco (1944), Tenorio (1947) and others. With pellets containing only 5 per cent of estradiol and 95 per cent of cholesterol and remaining for 4 or 8 months in a female guinea pig, fibroids can be elicited in some animals (fig. 34) although the incidence is greatly reduced. With pellets containing but

3 per cent of estradiol, there were at 4 and 8 months uterine and other abdominal fibroids in 6 out of 10 animals (Tenorio, 1947). As shown by the condition of the nipples, mammary glands and uterus, the estrogen was not exhausted at the end of these experiments. The following experiments also were very striking. The same pellet containing 5 per cent of estradiol was implanted successively in 4 animals remaining 4 months in each. Fibroids may be elicited even in the fourth animal (fig. 35). A pellet of 30 to 35 mg contained at the beginning but 1.5 to 1.7 mg of estradiol and this quantity, not being exhausted after 500 days of action, was capable of inducing fibroids subsequently in 4 different animals (Riesco, 1946, 1947). With the

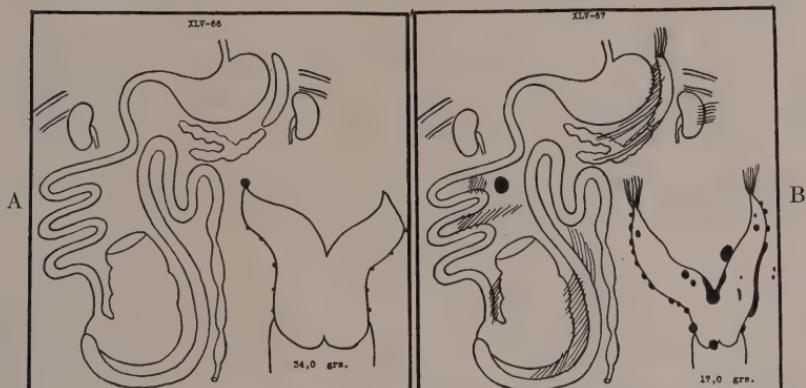


FIG. 35. Fibromatogenic action of minute quantities of estrad. from subcut. pellets. A. Pellet weighed 34.5 mg containing 5 per cent of estradiol, or 1725 µg of the hormone. The pellet was successively for 114, 124 and 110 days in three animals before implantation into animal XLV.66 in which it remained again for 121 days. Weight of uterus 34 gr (!). Small perimetrial tumors. Apical tumor on the right horn. B. Pellet originally 33.5 mg containing 5 per cent of estrad. or 1675 µg of the hormone. The pellet was for 114, 131 and 111 days placed successively in three animals before implantation into XLV.67 in which it remained again for 125 days. Weight of uterus 17 gr. Numerous uterine tumors. Mesenteric tumor and fibrous strands.

subcutaneous injections of the free hormone *no less* than 50 to 80 mg, instead of less than 1.6 mg, would have been necessary to obtain this experimental results in 4 animals.

Even more convincing as to non-selective absorption were experiments in which 1 per cent estradiol pellets which had remained in the body of other guinea pigs for as long as 2 years were again implanted into castrated guinea pigs. Opening of the vagina, growth of the nipples and increase of uterine weight beyond normal was obtained with these old pellets (Barahona, 1949).

One may calculate, though only roughly, the quantity of estrogen absorbed from fibromatogenic 3 or 5 per cent pellets. Absorption from pure

estradiol pellets having a surface of 65 mm² is of about 90 µg per day, or of about 1.4 µg per sq. mm (Lipschutz and Vargas, 1941c; Fuenzalida, 1950). The estradiol surface in similar pellets being reduced to 3 or 5 per cent, absorption, if not selective, will be only 2.7 or 4.5 µg per day. Indeed, it is probably much less than that, as the presence of cholesterol has been shown to diminish absorption even per sq. mm (Fuenzalida, 1950), and the above-mentioned experiment (Barahona, 1949) gives seemingly corroboration to it. A 1 per cent pellet of 35 mg contains but 350 µg of estradiol; allowing for an absorption at a rate of 0.9 µg per day, it should be void of any hormone at the end of the first year; but it contains estrogen even at the end of the second year!

As already emphasized, the incidence of fibroids diminishes greatly with quantities so small. There is seemingly in each group of individuals of a guinea pig population a gradient of sensibility to the fibromatogenic action of estrogens. With 1 per cent pellets no fibroids were produced.

B. THE COMPARATIVE HYSTEROTROPHIC AND FIBROMATOGENIC THRESHOLD CONCENTRATION. DIFFERENTIAL TUMORIGENIC THRESHOLD CONCENTRATIONS

The experimental evidence reviewed in the last three paragraphs leaves no doubt about the dynamics of the fibromatogenic action of the estrogen: *it is only by continuous action that estrogen acquires tumorigenic faculties.* It is also fully evident from our work that the sexual rhythm by which production and action of estrogen becomes intermittent is a safety measure against toxic and tumorigenic actions of the estrogens. One must suppose that rhythmic *diminution* of estrogen in the body may serve for the same purpose. Rhythmic interaction of progesterone which antagonizes certain actions of the estrogen also may serve for this purpose as we shall see in Parts II and III. Here we shall try to consider the quantitative aspects of the fibromatogenic action of estrogens, tentatively, i.e. without referring to the antagonistic actions of other steroids.

According to our concept the problem of the fibromatogenic *quantity* cannot be separated from the problem of the fibromatogenic *threshold concentration* of the hormone in the body. The concept as far as it refers to *concentration* is certainly hypothetical. But the results of our experimental approach are sufficiently clear as to two points: *first*, that the old concept according to which the toxic and tumorigenic actions of estrogens become manifest only when *large quantities* of the hormone are introduced in the body must be *definitely dropped*; and *secondly*, that no true understanding for the break from the physiological to the tumorigenic activity can be achieved without considering the *timing* conditions of the action of estrogens.

There is another quantitative aspect very relevant as to the dynamics of the fibromatogenic action of estrogens: the threshold concentration on whose continuous maintenance fibromatogenic action is supposed to depend, must it be higher than the concentration maintained by normal ovarian function? This question has been studied in the course of our work in different ways. The maintenance of a normal uterine weight in castrated females may be used as a test for the physiological ovarian function. Then the above question may be expressed in the following words: is the fibromatogenic threshold higher than the hysterotrophic one? The uterus can be maintained at a normal weight in a castrated female with injections of the free or esterified hormone in the course of several months in quantities so small that fibroids fail to appear. This result seems to show that the fibromatogenic threshold is higher than the hysterotrophic one.

Another finding also was in favor of this conclusion. As has been shown in preceding sections abdominal fibroids may be elicited with minute quantities of estrogen absorbed from subcutaneously implanted pellets (see p. 41). The uterus may attain with similar pellets in the course of 3 to 8 months the monstrous weight of 23, 36, 47 and even 60 (!) gr (figs. 34, 35). And nevertheless the incidence of fibroids is, with small quantities of the hormone, greatly reduced, as already insisted upon. Also with certain estrogens, as equilenin, dihydroequilenin, and with the derivatives of the dehydrodoisynolic acids which are less fibromatogenic than ovarian estrogens (see p. 32 and 41), one may often obtain enormous uteri without fibroids being elicited. One must suppose that the concentration maintained with these minute quantities of estrogen, though sufficiently high so as to cause such a great increase of the uterus, did not always reach the fibromatogenic threshold concentration as evidenced by the absence of fibroids.

The conclusion which has been reached, by our experimental work, about the quantitative conditions of the fibromatogenic action of estrogens can be resumed in the following statement: When there is a continuous flow of estrogen in the body due to the injection of very slowly absorbed esters of the hormone, or to a subcutaneously implanted pellet, the quantities of the hormone necessary for the maintenance of the fibromatogenic threshold concentration are certainly minute in comparison with those which are so often thought to be necessary for the experimental production of atypical tumoral growth. On the other hand, these minute quantities are already greater than those which are necessary for the maintenance of a normal uterine weight—or which are continuously produced in the normal ovary.

But here it must be strongly emphasized that these quantitative conditions of the *fibromatogenic* action of estrogen cannot be generalized. There is full evidence from our work that there are *differential tumorigenic threshold concentrations*. This may be exemplified, first, by the results already men-

tioned above which were obtained with the implantation of pellets of urinary estrogens or of the derivatives of the doisynolic acid. They are less fibromatogenic than ovarian estrogens under similar conditions of administration. But notwithstanding that there was, even in the absence of fibroids, considerable proliferation of the endometrium and of the uterine glands, adenomatous polyps filled the uterine cavity, and uterine glands penetrated into the myometrium as early as 75 days after the beginning of estrogenic action. Experiments also showed that the injection of quantities of ovarian or artificial estrogens will cause atypical epithelial proliferation at a level when no fibroids are as yet produced (see figs. 52A, 54D and 55B); or by injection of the highly fibromatogenic 17-caprylic ester of estradiol but in quantities so minute that fibroids are not yet elicited whereas there is already a highly pronounced atypical epithelial proliferation. It is, finally, exemplified also by implantation of pellets of estradiol mixed with cholesterol which allow for the absorption only of minute quantities of estrogen. Fibroids are not produced at all, or their incidence is very small, though the condition of the uterine epithelia is that of proliferation including penetration between the muscular layers (see fig. 56, 57, 58).

All this refers to the *guinea pig*. As shall be discussed more fully in ch. 6 the tumoral response to estrogen differs greatly according to the species. But it is remarkable that epithelia, throughout all the species examined, react more readily to estrogen with atypical proliferation than myo-conjunctive tissues of the genital tract.

Chapter 4.

THE PROBLEM OF LOCALIZATION OF THE FIBROMATOGENIC ACTION

Atypical proliferation from which the abdominal fibroid results, is elicited by an hormone, or morphogenic substance, when allowed to act continuously instead of intermittently as under normal conditions. The question arises why atypical proliferation is limited *territorially* though the substance is circulating in the organism. This question is fundamental for the concept of cancer and neoplasm in general.

The site at which abdominal fibroids were found in the course of our experiments in the last ten years was so variable that, at the first glance, one may have supposed that chance and not law governed localization of these experimentally induced tumors. On the other hand, experience has shown that typical sites of localization can be established, and the study of these localizations offers the opportunity to discuss the problem of tumoral localization in general.

A. TYPICAL SITES OF LOCALIZATION OF ABDOMINAL FIBROIDS

When small quantities of a fibromatogenic ester, say 5 μg of the estradiol benzoate or dipropionate, were injected thrice weekly, small tumoral nodules—the “tumoral seed”—appeared on the surface of the spleen and on such surrounding sites as the great curvature of the stomach and the abdominal wall including the diaphragm (see above). In two animals in which at necropsy *situs inversus* was found, with the spleen on the right side, tumoral seed was again present on the surface of the spleen and the surrounding *right* parts of the abdominal wall whereas fibroids were absent at other sites (Iglesias; Carrasco; not published). It is evident that the spleen, and by its intermediance the hypochondric region, are the first to react with small quantities of estrogen. From here the tumoral reaction seems to irradiate to the right affecting the serosa of the digestive and genital tract. Von Wattenwyl (1944, p. 112) was unable to corroborate our findings with the predominance of the left hypochondric region; this is probably due to his not having worked with graded quantities of the hormone. The phenomenon is best seen in injection experiments as mentioned above.

The tumoral seed on the spleen and on the serosa of the left hypochondric region is also the last to disappear when production of abdominal fibroids is prevented by the simultaneous administration of antifibromatogenic steroids (see Part II). The preponderance of the splenic localization is also very striking in the male guinea pig. There is a pronounced sex specificity as to the fibromatogenic reaction (see chapter 5). In most cases, even with considerable quantities of estrogen, large abdominal extragenital fibroids are absent in the male whereas the tumoral seed is mostly present also in the male.

It would be difficult to discuss the question of the real meaning of this prevalence of splenic localization. One might be inclined to think that it is

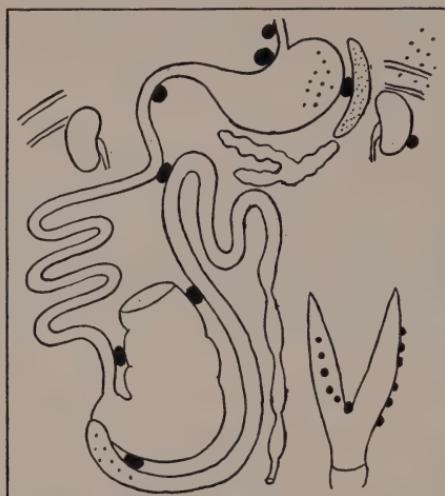


FIG. 36. Diagram showing the most important sites where fibroids are found in the abdominal cavity of female guinea pigs with the prolonged action of estrogens. "Angular" sites and sites of "mutual contact."

related to special metabolic conditions in the spleen or to a special sensitivity of the splenic serosa or subserosa. But then there is the contradictory fact that the parts surrounding the spleen, or being in intimate contact with the spleen, partake of the noted prevalence of the splenic tumoral localization.

The localization of the extrasplenic fibroids also seems to obey a certain rule. The diagram of figure 36 resumes a thousandfold experience of ours: the extrasplenic abdominal fibroids appear by preference at "angular" sites, or in sites of "*mutual contact*" of abdominal organs. By angular sites I mean the cardias, pylorus, the transition from the ileum to the cecum, and from the latter to the colon. Tumors appear with frequency in the angle between kidney and abdominal wall. Uterine tumors appear also in

the angle between the two horns (figs. 2, 34). The prevalence of the splenic localization may be due to contact between the spleen and the stomach, the diaphragm and the abdominal wall. Fibroids are frequent also where the large cecum almost meets with the colon ascendens. The localization of tumors on the prominent ventral ridge of the uterine horn also is remarkable (fig. 1B).

B. LOCALIZATION CONDITIONED BY ADJUVANT UNSPECIFIC MECHANICAL AND CHEMICAL STIMULI

Localization of experimental fibroids at "angular" sites or in sites of "mutual contact" necessarily suggests an *unspecific* mechanical influence. Such an influence has been shown to be real.

When a pellet of estradiol was put in close contact with the epiploon of female guinea pigs it became surrounded by tumoral growth (Chaume and Vargas, 1941). It was the same with pellets of estradiol benzoate fixed beneath the serosa of the uterus in the work of Perloff and Kurzrok (1941) whereas no tumoral growth was elicited when pellets of paraffin or of cork were used. Clear cut results were obtained in our work when the unspecific stimulus was introduced into the abdominal cavity and the estrogen allowed to be absorbed from a subcutaneous pellet.

A metallic pellet, the dentistry alloy of aluminium and copper, was used (Manhood, 1942). In animals not receiving estrogen the pellet becomes in general attached to the epiploon and surrounded by a thin fibrous capsule (fig. 72; ch. 13); it may be attached also to the peritoneum at other sites. When estrogens were administered the metallic pellet was found, about three months later, in an epiploic (fig. 37A), pelvic or parametrial tumor. Its fibrous structure was in general very similar to that of the estrogen-induced fibroid. By provoking a foreign-body-reaction the pellet determines the localization of an estrogen-induced fibroid, and acts as a local non-specific stimulus or "*path-maker*" for the specific stimulus (Manhood, Yanine and Lipschutz, 1945).

The superficial layers of the metallic pellet sometimes are transformed into a pulp. So the question arose whether with the metallic pellet a *mechanical* or *chemical* factor was at work. But glass beads, like the metallic pellet, also became the center of a tumoral localization in the epiploon or other parts when estrogen was present (fig. 37B; Montreal, 1944; Montreal, Iglesias and Lipschutz, 1945). These findings give definite evidence that localization of estrogen-induced abdominal fibroids may depend on the simultaneous action of an unspecific mechanical stimulus. But this statement is not sufficient to exclude the possibility of an interference of an unspecific chemical stimulus. Several metallic pellets, or several glass pellets, were introduced into the abdominal cavity of the same animal.



FIG. 37. Fibromatogenic reaction around a foreign body in the abdominal cavity.
A. Metallic pellet in a tumor of the epiploon. Tumor opened. Subcut. pellet of hexestrol; 93 days. (fem. G.p. LXIV.88). B. Large tumor of epiploon containing pellet of glass (arrow). 12 µg of hexestrol per day, subcut. pellet 202 days. (fem. G.p. LXIV.17).

There were 27 females with 45 metallic pellets; 87 to 190 days after the beginning of the experiments 29 out of the 45 pellets, or *two thirds*, were found fully involved in fibrous tumors. There were 20 females with 36 glass pellets, or beads, in the abdominal cavity; 88 to 202 days later only 13 out of the 36 pellets, or *one third*, were found fully enveloped in fibrous tumors. These comparative results show that local irritation by a chemical substance which by itself is unable to induce a fibroid may act as a localizing "path maker" for the latter when estrogens are allowed to act simultaneously.

Different former findings are in agreement with the above statements. Operative interference at different sites in the abdominal cavity favors the localization of fibroids. In castrated females to which estrogens are administered, large fibroids of the mesosalpinx, the so-called "apical" tumors, are very frequent (Iglesias, 1938) (figs. 3, 5, 8). On the contrary, in non-castrated females fibroids of the mesosalpinx are extremely rare (Murillo, 1940, and others) (fig. 42). In hysterectomized animals fibroids may appear at the site of the suture of the vaginal wall (Vargas; unpublished experiments). In males in which a ligature was made on the ductus deferens, tumors were elicited at the site of the ligature when estrogens were given (Koref, Jedlicky, Szabó, Palma et al.; fig. 44). These tumors were the most typical fibromyomas or myomas we have met with in our work with estrogen-induced fibroids (ch. 2, B, 2 and fig. 17).

Findings of other workers have to be mentioned here. Cauchoux (1939; p. 47-51) reported a significant fibrous reaction around a thread of cotton in the myometrium of animals receiving estrogen during several months; these "interstitial fibroids" were absent in animals not receiving the hormone. Von Wattenwyl (1944; p. 105-106) observed a fibrous tumoral thickening of the scar of the abdominal serosa in animals in which an incision of the ventral abdominal wall was made and estrogen was given during 2 to 4 months.

We were also fortunate to observe by chance the localizing action of an other unspecific stimulus which was undoubtedly chemical. Estrogen was administered to female guinea pigs which for other experimental purposes received intraabdominal injections of Congo Red. Small nodules were found in four out of five cases on the gall bladder which is a rare localization (Puentes and Riesco, 1945; Puentes, 1945). Microscopic examination showed a partial necrotization of the wall of the gall bladder. There was also in these animals a thickening and retraction of the mesentery of a degree probably never—or only very rarely—observed with estrogen alone (fig. 38).

Indeed, the above findings, though conclusive as to the localizing influence of unspecific mechanical or chemical stimuli, do not exclude the action

of other localizing factors. We may refer here to parametric fibroids related to the blood vessels (fig. 23, A and B).

It is of considerable interest that seemingly "unspecific" stimuli also may induce atypical proliferation of epithelia, or in any case accentuate the metaplastic response to estrogen of epithelial tissue. In a guinea pig in which a resection of the upper end of the uterine horn was made and estrogen was administered for 4 and a half months, an ectropium of the uterus was unexpectedly produced (fig. 39A; Koref and Lipschutz, unpubl.). The part of the endometrium which was in direct contact with the abdominal organs underwent transformation into stratified epithelium (figs. 39C



FIG. 38. Combined action of estrad. and Congo red on the peritoneum in the guinea pig. A. Mesentery in normal animal. $\times 23$. (LXXXV.64). B and C. Mesentery of animal with subcut. pellet of estrad. (absorption 26 μg per day), and 34 intra-peritoneal inj. of 2 cc. of 2 per cent aqueous sol. of Congo red, 91 days. $\times 10$. (LXXX.23). Enormous thickening of the subperitoneal layers (B); the collagenous tissue may replace completely the loose tissue between the two serosal layers (C). Note that A is at $\times 23$, and B, C at $\times 10$.

and D) whereas the glands beneath the stratified endometrium, or in the proximal part of the horn, were of the type common in estrogen-induced glandular hyperplasia (fig. 39B). In fig. 39D the upper layers seem to undergo epidermization (?). A condition of the uterine epithelium somewhat similar to, though not so pronounced as, that with ectropium may be found at the distal end of adenomatous polyps of the endometrium when prolapsing into the vagina. Local mechanical trauma has been reported to enhance also cystic glandular hyperplasia in rats pretreated with estradiol (Selye, Borduas and Masson, 1942); endometrial polyps may be induced in guinea pigs not receiving estrogen by inserting glass beads beneath the epithelium (Blandau, 1949).

Production of endometrial moles by the combined action of local trauma

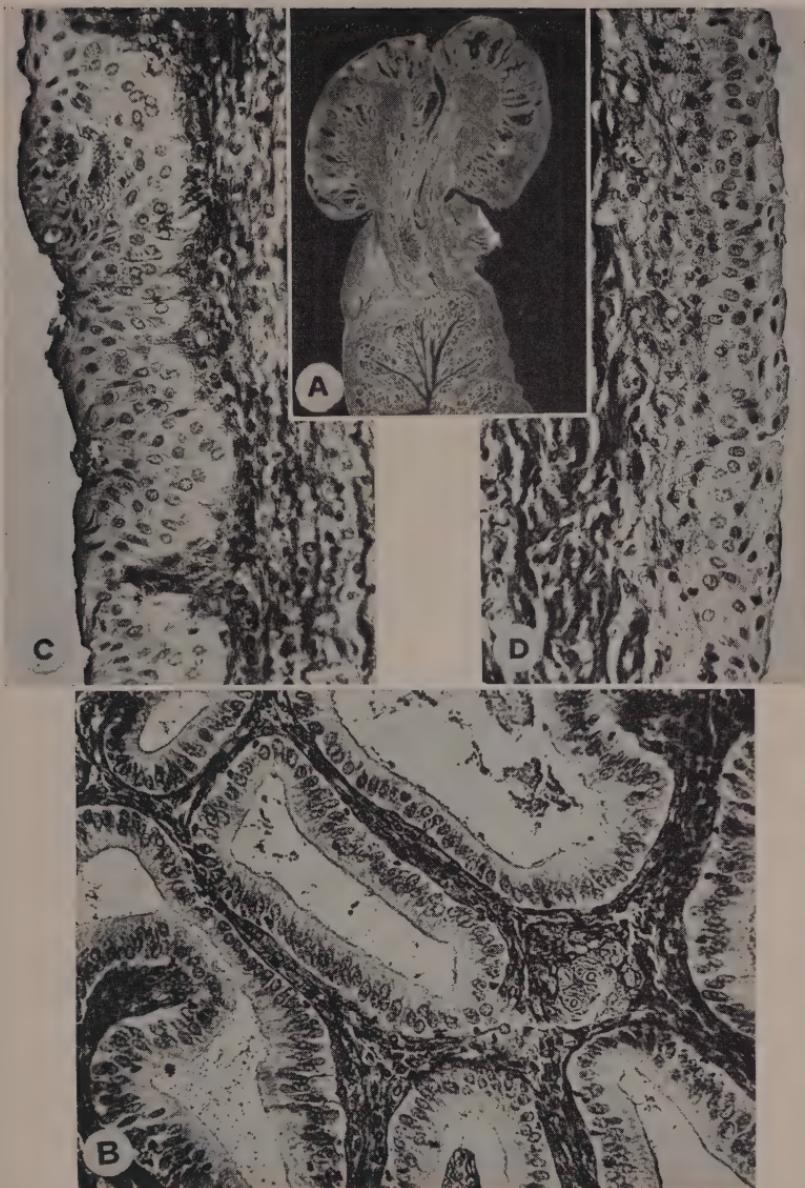


FIG. 39. Stratification of endometrium in ectropium of the uterine horn. 61 inj. of 80 µg of estr. (benz.). 142 days. (74/7). A. Frontal section through upper part of uterine horn. To the left, just between uterine horn and inverted part a subserous fibroid. The endometrium of the inverted part looks towards the abdominal cavity. Glandular cysts beneath the surface. $\times 3$. B. Cystic hyperplasia of uterine mucosa not in direct contact with abdominal wall with hypertrophic cells as common under the influence of estrogen. $\times 200$. C and D. Uterine mucosa looking towards the abdominal wall. Stratification, of a seemingly different aspect than in fig. 52.

and steroids (Selye, Harlow and McKeown, 1935; Selye and Friedman, 1940) also has to be mentioned here.

C. THORACIC FIBROIDS

Argentine workers reported fibroids on the thoracic surface of the diaphragm in 2 out of 30 animals with abdominal fibroids; the structure was the same as that of the abdominal fibroids (Sammartino and Herrera, 1940). In the work of Von Wattenwyl (1944; p. 112) there was 1 animal with a fibroid on the thoracic surface of the diaphragm; Mosinger (1946; p. 108, 134) reported fibroid nodules on the thoracic parietal serosa. In this Department thoracic fibroids were found, in the course of 10 years, only in 5 out of 1457 animals with estrogen-induced uterine or other abdominal fibroids (Bruzzone et al., 1948). There were tumors of the diaphragm but also parietal ones, with no connection at all with the diaphragm, but none of the pulmonar serosa (fig. 40A). A tumor was attached to the trachea (fig. 40B; R. Acuña, 1947). There were, besides the 5 animals mentioned, 6 others with small nodules just visible to the naked eye on the parietal or pulmonary serosa. Even when these 6 animals are added the incidence of thoracic fibroids is insignificant: a total of 11 animals with tumors or some fibrous reaction in a total of 1457 animals with fibroids in the abdominal cavity, or less than 1 per cent.

The question arises whether the differential incidence of abdominal and thoracic fibroids is due to a differential sensitivity of the endothelial or subendothelial cells of the two serosal covers, or to a factor present in the serosal fluid. Only the second of these possibilities has been so far investigated. An artificial communication between the abdominal and the thoracic cavity was established by partial excision of the left diaphragm (Elgueta, 1944; Elgueta, Iglesias and Bruzzone, 1948; Bruzzone, Elgueta et al., 1948). A subcutaneous implantation of pellets of estradiol or stilbestrol was made. A fibrous tumoral reaction was found 3 to 6 months later in the thoracic cavity of 7 out of 18 animals. In several cases the thoracic tumoral reaction was not limited to the left side communicating with the abdominal cavity; tumors appeared also on the right side. The tumors were mostly those of the thoracic serosa and, twice, of the diaphragmatic serosa also. In one case there was a small nodule on the mediastinum and in another case on the pericardium. The structure of these tumors was the same as that of abdominal fibroids.

There was in all animals a prolapse of the liver and of the intestine into the thoracic cavity; thus an unexpected interference of a mechanical stimulus might have been in play, in any case on the left side. But as to the right side this mechanical interpretation scarcely would apply. In animals without a communication between the thoracic and abdominal cavity a

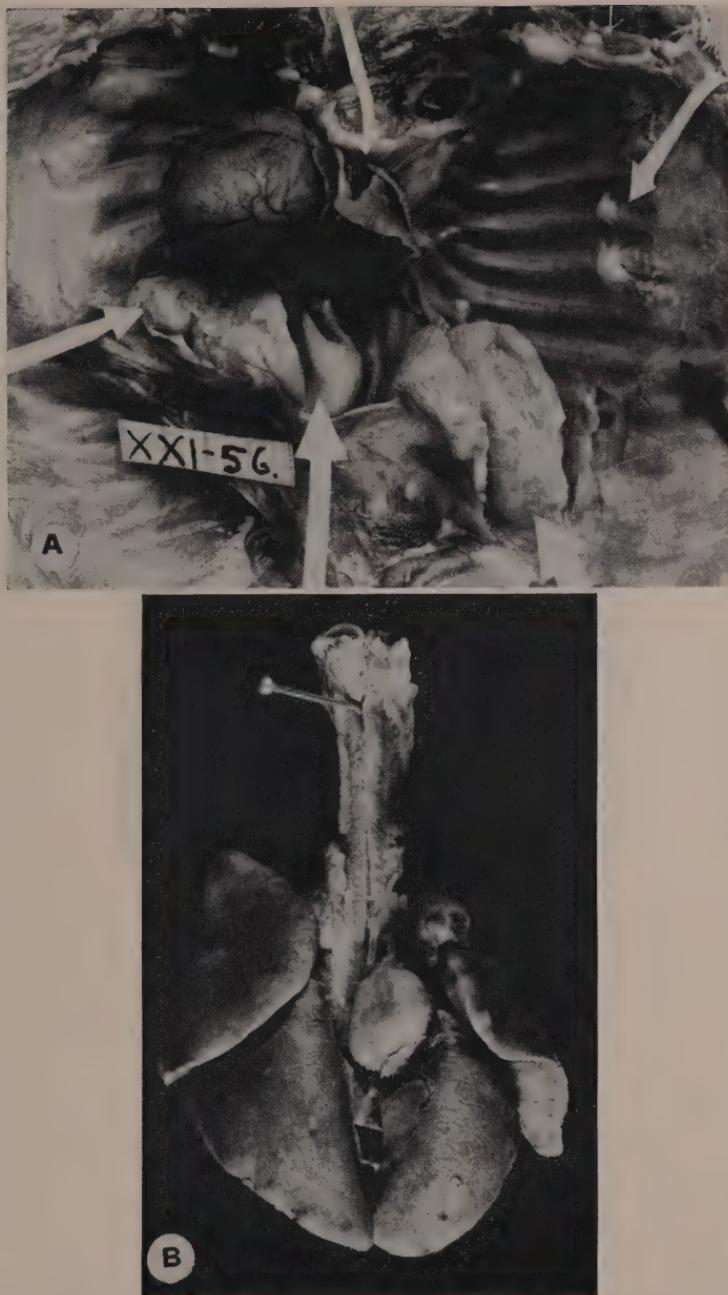


FIG. 40. Fibroids of the thoracic cavity. A. Female guinea pig. 91 inj. of $10 \mu\text{g}$ of estrad. (capryl.), 218 days. Various large tumors on the thoracic surface of the diaphragm. Smaller fibroids on the parietal pleura (left). Minute nodules on the lung. Size 1.1. (XXI.56). B. Male guinea pig. Dorsal view. The pedunculated tumor is attached to the trachea. Subcut. pellet of stilbestrol. 813 days. Size 1.3. (XCIII.58).

tumoral reaction was not enhanced by introducing a metallic pellet into the thoracic cavity. On the contrary, there was a very strong tumoral

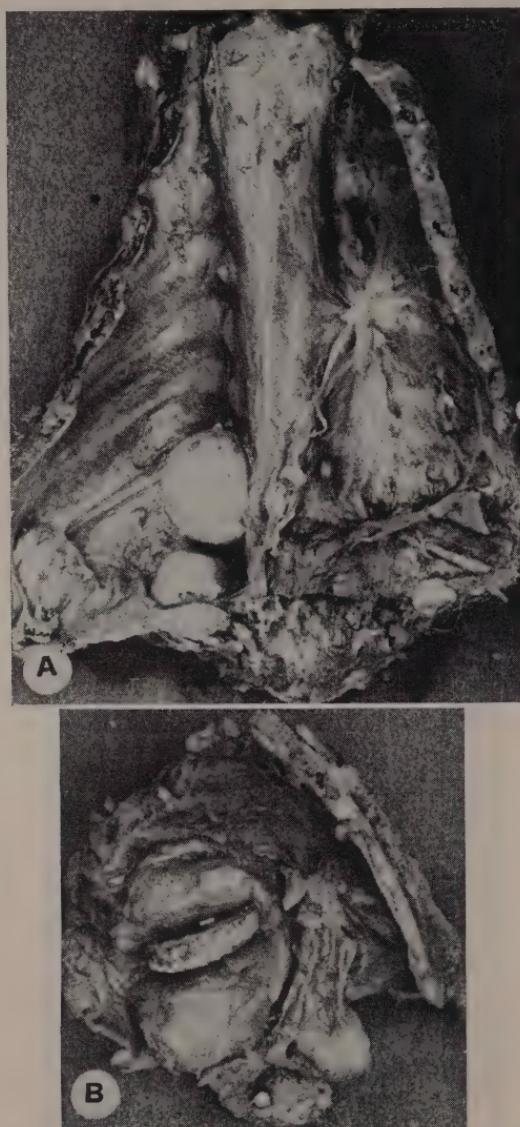


FIG. 41. Thoracic fibroids in female with opening between abdominal and thoracic cavity on the right side. Absorption of 8 μg of stilbestrol per day, subcut. pellet, 167 days. A. Two fibroids of the right parietal pleura and one on the diaphragm. Large tumor and fibrous strands of the left thoracic cavity. B. Large tumor of the left cavity; tumor opened and metallic pellet therein. (LXXIII.60).

reaction in an animal with an abdominal-thoracic communication on one side, and a metallic pellet on the other side (fig. 41).

One may thus assume that the estrogen-induced fibrous tumoral reaction of the peritoneum is mediated by a factor normally present in the abdominal serosal fluid, or a factor originating under the influence of the estrogen and secreted into the abdominal cavity. The absence of this mediating factor in the thoracic cavity would explain the very small incidence of estrogen-induced thoracic fibroids. After all it seems evident that localization of estrogen-induced tumors of the serosa depends on many factors. It is to simplify matters, or to hide our ignorance, that we term them "unspecific" stimuli. Indeed, we are very far from being able to settle these problems of localization to which a real interest must be attached from the point of view of neoplastic growth in general.

Chapter 5.

SEX SPECIFICITY OF THE ABDOMINAL FIBROMATOGENIC REACTION

For many years various authorities have directed attention to an increase of fibrous tissue taking place in different organs in animals subjected to the prolonged action of estrogens (see review of Zuckerman, 1940). Similar findings have been reported for the uterus of mice, rats and rabbits (Courrier; Lacassagne, 1935; Loeb et al., 1938a), and especially for the seminal vesicles in the rat (Freud; De Jongh; Korenchevsky; et al.). An increase of fibromuscular tissue in the prostate of the dog and monkey also has been described (Parkes and Zuckerman; Courrier and Gros; references in the exhaustive summary of Zuckerman, 1940). The fibrous strands and extended fibrous indurations found in guinea pigs on the parietal and visceral serosa of the abdominal cavity have already been mentioned (ch. 2, C). Their microscopic structure is somewhat different from that of fibroids; fibroblasts are rare and collagen fibers predominate; the disposition of the fibers is in fibrous strands a more orderly one than in fibroids (Lipschutz and Vargas, 1941a).

These statements are of interest because they suggest as already insisted upon (p. 30) that experimental abdominal fibroids including uterine fibromyoma are but an especially pronounced local manifestation of a general pathological response of conjunctive tissues to the continuous action of estrogen.

As extrauterine fibroids can be elicited by estrogens in the female the question arose whether similar tumors would originate also in the male. The response of the male guinea pig, castrated or noncastrated, was different from that of the female (Koref et al., 1939; Jedlicky et al., 1939; Szabó, 1940; Chaume, 1940; Lipschutz, Vargas and Palma, 1941; Palma, 1940). Under the same experimental conditions under which extrauterine fibroids are induced in almost every female, these tumors are elicited only exceptionally in the male. The reaction characteristic of the male (figs. 42 and 43A) is the tumoral seed on the spleen and the surrounding parts of the stomach and the abdominal wall; there may be also an ample disseminated fibrosis on the abdominal wall and the mesentery. Small nodules may appear on the serosa of the testicles, of the kidney and even on the

visceral serosa, especially when the experiment is prolonged; in these experiments the fibrous reaction may become highly pronounced spreading

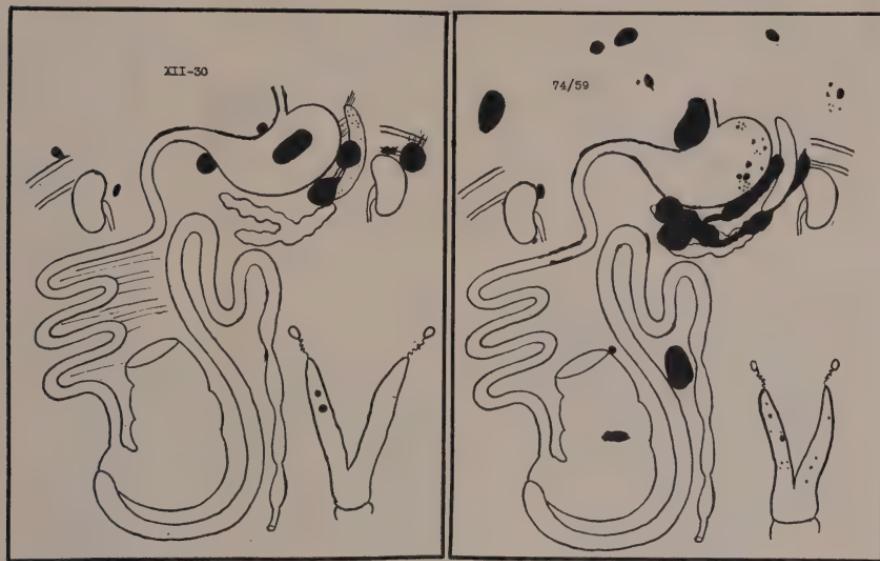


FIG. 42. Non-castrated female guinea pigs. A. 40 inj. of 10 µg of estrad. (capryl.), 90 days. Uterine and extrauterine tumors. No apical tumors (XII.30). B. 61 inj. of 80 µg of estrad. (benz.), 141 days. No apical tumors. (74/59).

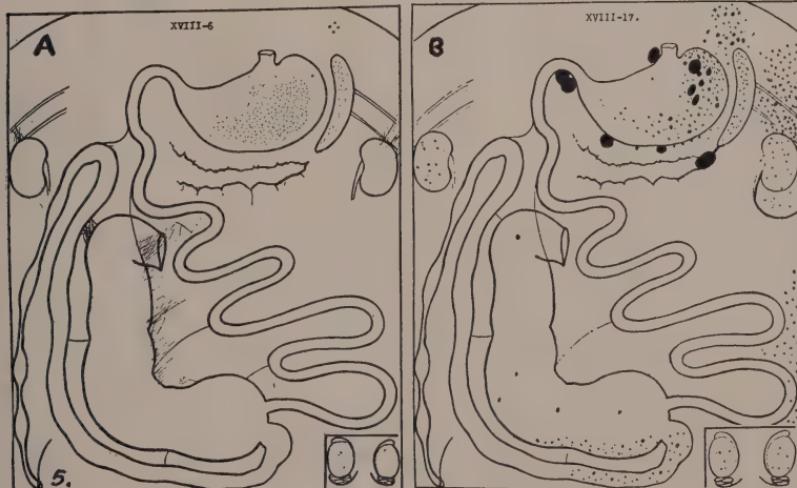


FIG. 43. Non-castrated male guinea pigs. A. Typical reaction. Small nodules on the tunica albuginea testis. 38 inj. of 80 µg. of stilbestrol, 87 days. (XVIII.6). B. Non-typical reaction: appearance of large fibroids as in the female. 38 inj. of 320 µg of stilbestrol, 88 days. (XVIII.17).

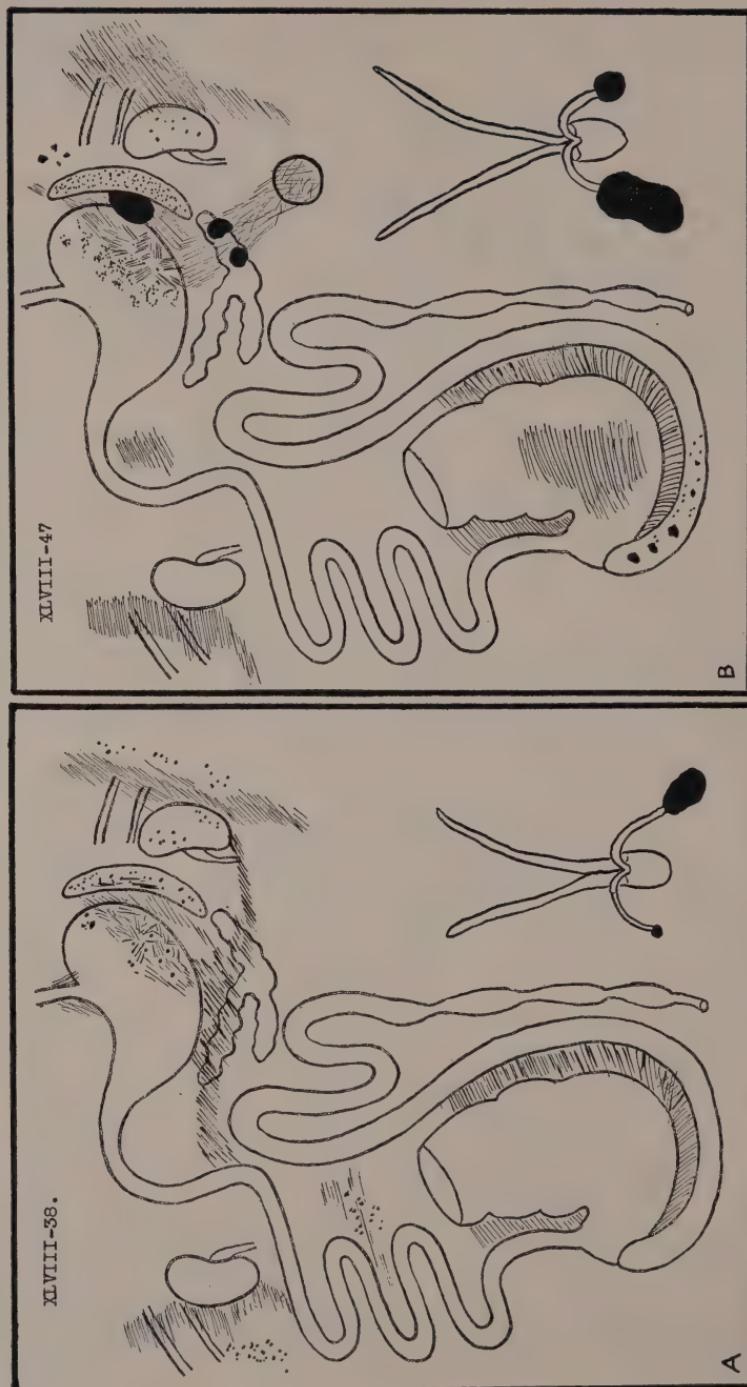


FIG. 44. Male guinea pig; very prolonged administration of estrogen. A. 279 days. (XLVIII.38). The reaction is still typical of the male: tumoral seed of the spleen and adjoining regions of the stomach, abdominal wall and diaphragm, but spreading also to the right. Inguinal tumors. Tumor of the prostatic region. B. 275 days. (XLVIII.47; see also fig. 45B). Abdominal tumors.

to everywhere in the abdominal cavity. The tumoral seed may appear also in the right hypochondrial region. The fibrous strands may become ample and thick. Spherical fibroids also may appear (figs. 43 to 45), and sometimes as early as at 6 weeks of estrogenic action (Torrico, unpubl.). But even in experiments lasting 4 to 9 months fibroids similar to those in the female remained scarce in the male (Yanine, 1943). Thus in a group of 17 castrated males with subcutaneous pellets of estradiol dipropionate and an absorption of 17 to 40 μg per day there were at 112 to 160 days only 2 with large tumors.¹ In a group of 14 castrated males with an absorption of 17 to 31 μg per day there were at 275 to 283 days again only 2 with similar tumors



FIG. 45. Fibrous reaction of spleen and stomach in males, in experiments of long duration. A. Thick fibrous strands and tumoral seed on the great curvature of the stomach. Tumoral seed on the surface of the spleen. Tumor between spleen and stomach. 40 μg of estrad. (diprop.) per day. 16 days. (XLVIII.34). B. Similar reaction. Metallic pellet in thick capsule between stomach and abdominal wall which also shows a considerable fibrous reaction. 22 μg per day, 275 days. (XLVIII.47).

though the disseminated fibrous reaction and the tumoral seed attained in most of these animals a very considerable degree (figs. 44 and 45). Under these quantitative conditions there would scarcely be a female without large tumors. With very considerable quantities of estrogen the incidence of tumors increases in the male (Toro, 1947) but is always still much behind what one finds in females.

Results of Sammartino and Herrera (1940) were in full agreement with ours as to the sex different fibrous reaction, whereas Von Wattenwyl

¹ When comparing the fibrous reaction in the abdominal cavity of females and males only tumors of the digestive tract and the abdominal wall can be considered, and not those of organs particular to the sex as the uterus and the parametrium, or the ductus deferens and the prostatic region.

(1944, p. 116–122) did not notice any sex difference; he ventures that this was due to his experiments not having been sufficiently numerous.

How the difference of the tumoral response of males and females can be explained? At first sight the difference seems to be only a quantitative one. The same type of reaction, i.e. the tumoral seed on the spleen, the disseminated fibrosis in absence of tumors, can be seen also in the female when small quantities of estrogen are given, or when the fibromatogenic action of the estrogen is counteracted by other steroids. But this quantitative explanation does not hold against the fact that the type of response is only exceptionally altered in the male when treatment is prolonged.

When a metallic pellet is introduced into the abdominal cavity of a male and estrogen is administered there is no tumorous thickening of the surrounding capsule as in the female. Only in experiments of long duration, the fibrous capsule may become thickened (see fig. 45 B). The tumoral seed may appear on the capsule. But even in these cases the difference between male and female was mostly very striking.

The sex specific difference of the fibrous response to estrogens must be due to some intrinsic factor linked with the sex. One may refer first to sex specific *humoral* factors. The testicular hormone is under certain quantitative conditions able to counteract the fibromatogenic action of estrogens (Part II). But an interference of the testicular hormone is out of question since the sex specific fibrous response is in *castrated* males so pronounced as in *non-castrated* ones. One may then suggest that there is in play some *extragonadal* sex specific humoral factor whose existence has been demonstrated by our comparative work with ovaries grafted into castrated female and male guinea pigs; follicular development in the male never proceeded until corpus luteum formation, while cystic follicles persisted for a long time. Similar statements have been made by many authorities and in different species. I suggested that *extragonadal* sex specific factors or substances ("substances Y") were produced by some endocrine gland acting on the graft (Lipschutz, 1927b). In later years, after the gonadotrophic function of the hypophysis was discovered by Smith and Engle, and by Zondek and Aschheim, considerable differences between the male and female hypophysis have been shown to exist in the rat (references see Martins, 1936a; Lipschutz, 1942d). One may then tentatively suggest that some sex specific hypophysial factor is responsible for the differential fibrous response of males and females to estrogens. But the hypophysial sex differences as to the weight of the anterior lobe and content of luteinizing hormones (Lipschutz, 1936), cytology (Wolfe et al., 1937–1941), and the gonadotrophic faculty *in situ* (Pfeiffer, 1936) have been shown to depend on the previous action of gonadal hormones on the hypophysis.

Fibroids can be elicited by estrogens also in the hypophysectomized

female guinea pig (Vargas, 1943a). Unfortunately experiments in hypophysectomized *male* guinea pigs are still lacking; they would be liable to settle the question about the relevance, or non relevance, of hypophysial factors for the sex specificity of the fibrous response.

Whichever the explanation of the differential response of male and female may be, it is evident that we meet here with one of the most fundamental problems of neoplastic growth in general: the *differential response of homologous territories*. We shall deal with this problem in the following chapter.

Chapter 6.

SPECIES DIFFERENCES OF THE TUMORAL RESPONSE TO ESTROGENS

A. ABDOMINAL FIBROIDS

Attention was given in our work to the question whether abdominal fibroids would be produced in other species also. Six different species have been studied in experiments of long duration—the rat; two non domesticated cavies of Bolivia; the Andean rodent *Octodon degu*; *Macacus Rhesus*; and *Cebus apella*, or the Capuchin monkey from Ecuador.

The highly fibromatogenic 17-caprylic ester of estradiol by which abdominal fibroids are so easily induced in the guinea pig in the course of 3 months with 3 injections of 5 μg per week revealed to be inactive in the *rat*, and even then when as much as 80 μg per injection were given (Egaña et al., 1941). Fibrous strands also were absent in the *rat*. Neither were we successful with the subcutaneous implantation of pellets of stilbestrol in female rats which were necropsied after four months of treatment (Bruzzone and Febres; Febres, 1944). We tried in the *rat* also to combine the prolonged action of estrogens with the local action of a metallic pellet introduced into the abdominal cavity, a method so successful in the female guinea pig (see chapter 4, B). But we achieved no success in these experiments with the *rat*.

Production of uterine fibroids has been reported in rats under a diet deficient in vitamin E; it has been also reported that concentration of estrogen in the blood increases under these dietary conditions (Barrie, 1938; Martin and Moore, 1939; Shute, 1944; quoted from Burrows and Horning, 1947). In work with guinea pigs administration of vitamin E (Szabó, 1940) and of large quantities of vitamin A (Geldres, 1942) did not counteract the fibromatogenic action of estrogen. A fibroid rich in collagenous fibers and poor in nuclei has been reported by Kaufmann and Stein-kamm in a single rat to which 5 μg of estrone per day were administered in the course of 325 days (Arch. Gynaek. 165: 358. 1938; quoted from Alfieri 1938, p. 11).

In a group of 82 female mice, hybrids of C3H x PM (Andervont), which had not received any treatment, uterine fibroids or fibromyomata were unexpectedly found in 5 animals (Pan and Gardner 1948). See also the findings of Strong in mice receiving methylcholanthrene (ch. 9, D, 2) and of Pfeiffer in mice with an experimentally induced hormonal imbalance (ch. 18).

The Andean rodent *octodon degu* which is easily available in our country offered more interest than the *rat* because this rodent is, as to reproduction, very similar to the guinea pig. The young are born almost as developed as

in the latter. Administration of diethylstilbestrol was prolonged in 21 females for six to eight months without abdominal fibroids being produced; neither was there any other fibrous abdominal reaction visible to the naked eye (Febres, 1944).

Of special interest seemed experiments in wild South American cavies, i.e. *non-domesticated guinea pigs*. As has been shown by Detlefsen (1914; quoted from Von Uebisch and Mello, 1940) the wild Brazilian guinea pig *cavia aperea* can be crossed with the common domestic species; the offspring are very fertile. More recently these statements were corroborated in the native Brazilian habitat of the wild species (Von Uebisch and Mello, 1940). Wild cavies live also in the steppes of Argentine and in Bolivia. Dr. Iglesias went to the last mentioned country and was able to realize experiments on more than hundred females and males caught in the region of Cochabamba at about 8000 feet.¹ The experiments were prolonged for 7 months but without abdominal fibroids being produced.

In the *rabbit* uterine fibromyomas have been reported by Horning (1941). The tumors appeared 7 to 8 months after subcutaneous implantation of pellets of estradiol benzoate. In male rabbits receiving estrogen for almost two years there was a fibroid of the epididymis, fibrosis of the kidney and mesentery but no abdominal fibroids (Bern, 1949).

In the capuchin monkey *cebus apella* experiments were made on 9 females, castrated or non-castrated, and on 3 castrated males (Iglesias and Lipschutz, 1947; Bruzzone and Awad; Awad, 1946). Treatment with estrogens was continued for more than 500 days. Highly active esters of estradiol as the 17-caprylic and 3-n-butyric-17-benzoic esters, or the highly active propionic ester of benzestrol were administered. There were no abdominal fibroids. In 1 case there were, after 14 months of continuous action of estrogens, excrescences on the surface of the spleen; they were recognized on microscopic examination as being due to a proliferation of the splenic serosa. Some fibrous strands seemed to be present in the abdominal cavity of other animals also; but they were never very conspicuous and we did not feel sure about them.

In *rhesus* experiments were made on behalf of our Department by Vargas (1943b) in Corner's laboratory, and independently by Engle et al. (1943). No abdominal fibroids or other signs of a fibrous reaction were found. Some fibrous strands in Vargas' work with castrated animals were possibly due to the operative interference. No abdominal fibroids have been noted in the work of Pfeiffer and Allen (1948) though estrogen was administered to some animals for 3 to 5 years.

¹ In former writings of ours reference was always made to *Cavia aperea*. Dr. G. H. H. Tate, Curator of the Am. Mus. of Nat. Hist. in New York, has been so kind as to classify skins and skull of two animals of Dr. Iglesias' series. They were recognized as specimens of the caviars *Galea mustelooides* and *Caviella niata*.

It is evident from the above that the estrogen-induced uterine or abdominal fibroid is limited to the domesticated guinea pig and rabbit. In none of four other rodents and in none of two anthropoids were abdominal

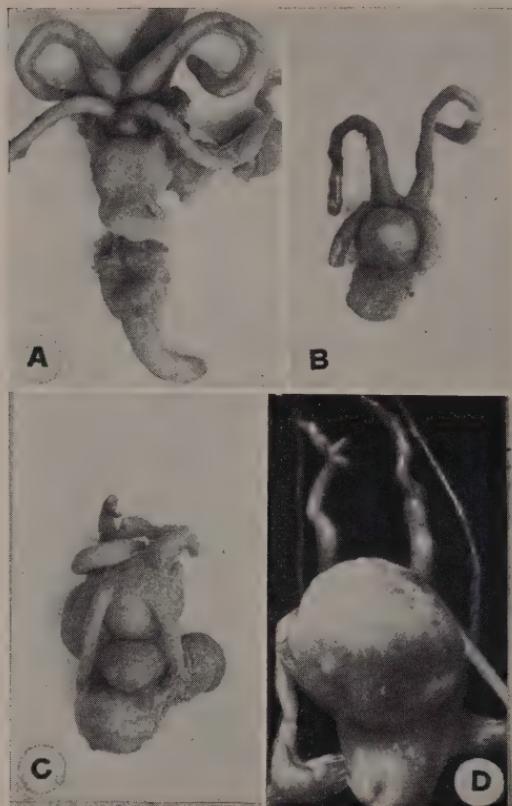


FIG. 46. Tumors of the utricular region in male guinea pigs. All figures nat. s. ze. A. 282 days: small tumor in the triangle between the deferent ducts. Fibromyoma with infiltration of epithelial structures due to proliferation of the utriculus as exemplified in fig. 48. About 20 µg of estrad. per day, subcut. pellet of diprop. (male G.p. XLVIII. 49). B. 164 days: about 4 µg of hexestrol per day, subcut. pellet (male G.p. LIII.S.8). C. 334 days: tumoral masses between deferent ducts and urinary bladder. About 5 µg of hexestrol per day. (male G.p. LIII.S.1). D. 275 days: 12 µg of estrad. per day, subcut. pellets of diprop. Beneath the tumor—urinary bladder. Nat. size. (XLVIII. 65).

fibroids induced under experimental conditions which with the guinea pig give an incidence of fibroids or fibrous strands in 80 to 100 per cent of the animals. Indeed, our observations in *Cebus apella* reported above show that species differences—like sex differences—are in some instances probably of a quantitative order only. But it remains true that the cells of the

peritoneal cover show a differential response according to the species. It is the same with another kind of tumorous reaction: the estrogen induced fibromyoepithelioma of the prostatic region of the guinea pig.

B. UTRICULAR FIBROMYOEPITHELIOMA

Estrogen-induced hyperplasia of the fibromuscular tissue of the prostate, metaplasia of the utricular mucosa and other parts of the prostatic region have been described by eminent workers in various species especially in mice, dogs, monkeys and including the guinea pig (Courrier and Gros; Lacassagne; De Jongh; Von Wagenen; Parkes and Zuckerman; Burrows; Kennaway; et al.; summary Zuckerman, 1940; new work on the rabbit of Chevrel-Bodin and Leroy, 1941, and Bern, 1949; for the guinea pig La-



FIG. 47. Tumor of seminal vesicles. Mostly fibromyomatous. 11 μ g of stilbestrol per day, subcut. pellet, 734 days. Nat. size. (XCIII.69).

queur 1936, Courrier and Cohen-Solal, 1936). The authorities did not refer to "tumors," though there was the statement that the prostate may increase in dogs treated for 6 weeks with estrogen as much as 5 and even 8 times (Kok, 1936); a very considerable increase of the prostate was also seen by J. R. Valle (Fac. of Med., San Paulo) in a dog treated during one year with estrogens (pers. communic.).

In our work tumors of the prostatic region have been elicited by natural and artificial estrogens in castrated or non-castrated males in experiments which lasted seven to eleven months (figs. 46 and 47; Yanine, 1943; Lipschutz, Yanine et al., 1945). Though these tumors may appear already as early as three months and a half after beginning to administer estrogens and even earlier than that (fig. 48; Montreal, 1944) this is rather exceptional. At 105 to 182 days the tumor was found in 7 out of 61 animals; at 204 to

342 days the tumor was found in 25 out of 60 animals, the incidence having increased from 11.5 per cent to 42 per cent. In experiments of long duration the tumors attain a considerable size (fig. 46C and D; J. Acuña, 1944; Silberman, 1944; Echániz, 1944; Lermandá, 1945; R. Acuña, 1947). The tumor of this region has been reported also by Von Wattenwyl (1944, p. 119) as prostatic, and by Mosinger (1946, p. 142), in one case, as utricular.

The utricular fibromyoepithelioma is due, in the first place, to hyperplastic growth of the fibromuscular periutricular tissue. There is besides

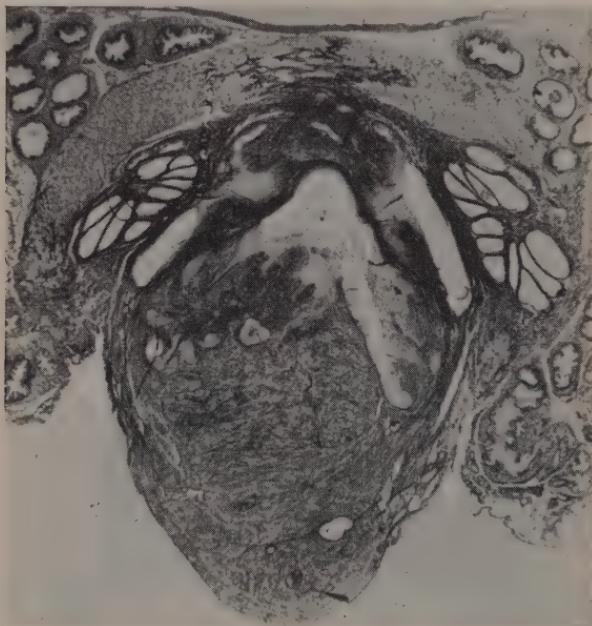


FIG. 48. Fibromyoepithelioma of the utricular bed. Utricular cavity enlarged. Epithelial proliferations penetrating deeply into ventral fibromyoma. Lateral to the utriculus the deferent ducts, transformed into solid epithelial cords, not yet separated from the ejaculatory ducts. 8 µg of stilbestrol per day, subcut. pellet, 108 days. $\times 10$. (G.p. LXIV.3).

this metaplastic growth of the mucosa of the utriculus masculinus and of a limited area of the vasa deferentia (Lipschutz, Yanine et al., 1945). The epithelial proliferations are found embedded in the fibromuscular tissue which they invade deeply, at a considerable distance from the utriculus (fig. 48). The metaplastic changes of the utricular mucosa are of two different types as epidermization and mucification. Both may be present simultaneously or they may intermingle. Sometimes the epithelial proliferation prevails and the tumor may consist preferably of solid masses of epithelial tissue (fig. 49). The tendency to epidermization does not mean

that regular or typical epidermis is produced; epidermization presents itself in a distorted form, and the atypical tissue organization is very striking. In some cases epithelial excrescences of the utriculus become cystic. The cysts may be lined with an epidermis with a thick cornified layer. In other cases the cysts are filled with papillomatous growths. In older tumors, i.e. in experiments of about two years duration or more, the epithelium

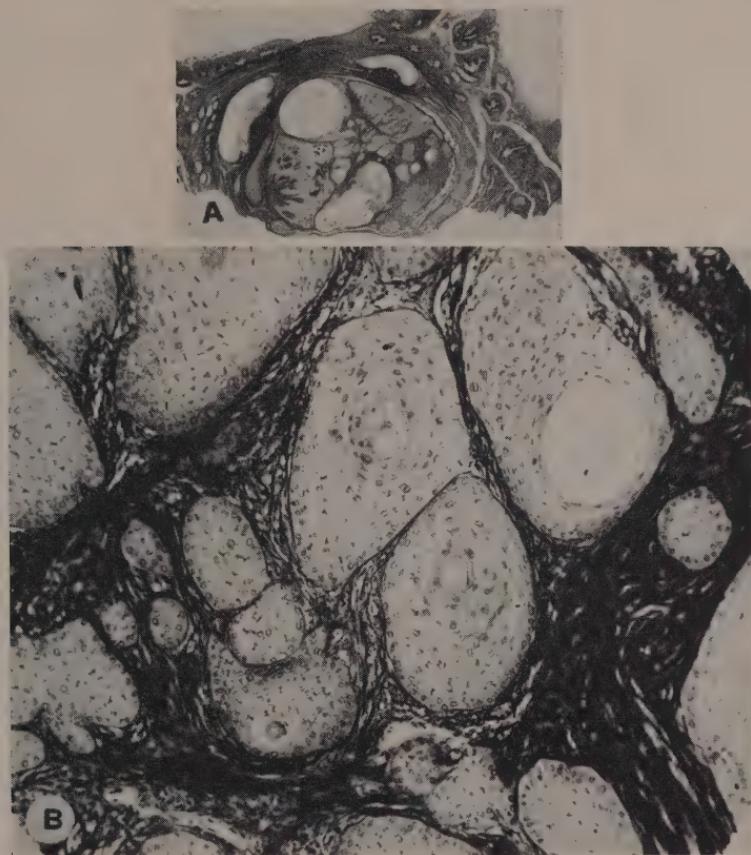


FIG. 49. Tumor of the utricular region consisting preferably of epithelial proliferations. 15 µg of estrad. per day, subcut. pellet of dipropion., 280 days. A. Laterally—ducts of seminal vesicles and prostate. Deferent ducts not identified. Cystically enlarged utriculus; the other cyst probably enlarged deferent duct (?). The remaining masses are epithelial proliferations. $\times 5$. B. Epithelial cords. Concentric disposition of cells. $\times 90$. (G.p. XLVIII.42).

may disappear more or less completely by necrotization. The cysts are then filled almost entirely with queratinized masses and only remains of pycnotic epithelial cells betray the origin of these encapsulated masses

amidst the fibromyoma. The latter may undergo an extensive sclerotic transformation.

The tumor of the prostatic region, like the abdominal fibroid, offered several aspects which were of interest from the point of view of neoplastic growth in general.

(1) The fibromyomatous part of the utricular tumor was structurally different from the estrogen-induced fibroid of peritoneal origin. In the latter, as we know, fibroblasts may prevail, the quantity of smooth muscle tissue being variable and often small. On the contrary, the non-epithelial part of the utricular fibromyoepithelioma was almost always a myoma, or fibromyoma, with epithelial ingrowth. The difference was especially striking when there were additional tumors of peritoneal origin around the basis of the seminal vesicles, or on the urinary bladder. These were typical abdominal fibroids in which myomatous tissue may also indeed prevail as was the case in figure 47 where the tumoral mass became very considerable with the long duration of the experiment. A fibrous nodule of the seminal vesicles has been reported also in a rat which had received a total of 15 mg of estradiol benzoate in the course of 10 months (Del Castillo and Sammartino, 1937).

(2) The hyperplastic and metaplastic phenomena of the prostatic region from which the estrogen-induced utricular fibromyoepithelioma derives in the male *guinea pig* are fundamentally coincident with the changes occurring in the male accessory organs of the prostatic region to which reference has been made above and which have been discovered by former workers in other species including the guinea pig. But so far the tumor as described above has been found only in guinea pigs. We were unable to induce the tumor in the Capuchin monkey. Our work with the guinea pig and the mentioned work in dogs, especially the finding of Valle, make it probable that *time* is of fundamental importance in this type of tumoral response.

The dependence of prostatic tumoral growth on hormones has attracted considerable interest in human pathology. Since there is in benign prostatic hyperplasia also adenomatous proliferation it has been assumed that androgens also must be responsible for this growth (R. A. Moore, 1947; with references). In a patient who was treated for several months with stilbestrol on a clinical diagnosis of prostatic cancer, the prostate showed squamous metaplasia both of the glandular epithelium and of the utricular one; the histological picture was quite unlike that of benign prostatic enlargement (Inglis, 1948). But on the other hand it has been claimed that the adenomatous proliferation in benign prostatic enlargement does not originate from prostatic glandular tissue but from the periurethral glands, and the latter have been suspected to be of mullerian origin like the female

genital tract; they are absent in dogs and anthropoids (summary Hinman, 1947). I am unable to give an opinion in this special problem.

C. ATYPICAL EPITHELIAL PROLIFERATION IN THE HYPOPHYSIS

Differential responses of zoological species to the continuous actions of estrogens have been studied also in other parts of the body.

It is well known that in rats and mice the anterior lobe increases very considerably and even monstrously when the animal is subjected to a prolonged treatment with estrogens (Cramer and Horning, 1936; McEuen, Selye and Collip, 1936; Zondek, 1936, 1938; Weil and Zondek, 1939; Egaña et al., 1941; summaries Selye, 1947b, and Dux, 1948). The hypophysis of the rat increases enormously under the influence of estrogen even when grafted into the anterior chamber of the eye (Martins, 1936b).

Most of the workers have used large quantities of estrogen. In our work hypophysial increase has been produced also with minor quantities. Rats were injected thrice weekly during three months with 10 µg of estradiol given as the 17-caprylic ester. With this quantity—a total of only 400 µg of estradiol in 3 months—the average hypophysial weight, which in normal males and females was 2.5 and 4 mg respectively per 100 g body weight, increased to an average of 14 to 15 mg. With quantities as great as 80 µg per injection—or a total of about 3000 µg in 3 months—an average of 40 mg per 100 gm body weight may be obtained. The hypophysis may reach the weight of almost 200 mg as against 8 to 10 mg in a normal animal. But a hypophysial weight of 35 mg may be obtained already with quantities as small as 2 to 4 µg per injection, or a total of only 100 to 150 µg administered in the course of 3 months (Egaña et al., 1941). The longer estrogen is administered the greater is the increase (Deanesly, 1939a; Noble and Collip, 1941b; Nelson, 1941).

The question may be raised whether this estrogen-induced increase of the hypophysis due to proliferation of the cells of the anterior lobe is to be called tumoral. Different authorities refer to estrogen-induced "chromophobe adenoma." In some cases, indeed, the whole anterior lobe is transformed into a highly vascularized mass of chromophobe cells; but in other cases the proliferation is a nodular one (Wolfe and Wright, 1938; see summary of Dux, 1948). There may be an unusual number of mitotic figures and polynuclear giant cells (Selye, 1944).

Spontaneous tumors of the anterior lobe are a frequent occurrence in old rats varying according to the strain; an incidence of 25 to 68 per cent has been found in animals more than 20 months old (Wolfe, Bryan and Wright, 1938; Oberling et al., 1939); the spontaneous tumors are transplantable (Saxton, 1941; Saxton and Graham, 1944) whereas with experimental tumors the reports as to this are contradictory (see chapter 8). Different

authorities have published exhaustive cytological studies of the spontaneous tumors. They were, like the experimental tumors, mostly chromophobe adenomatous growths notwithstanding the considerable polymorphism as to cytological details (Dux, 1948). In mice spontaneous hypophysial tumors are extremely rare (Selye et al., 1921).

There is the fundamental fact that the estrogen-induced increase of the anterior lobe is limited to certain species as rats and mice, and even in these species the reaction varies according to the strain (Lacassagne and Nyka, 1937; Gardner and Strong, 1940; Gardner, 1941b; Segaloff and Dunning, 1945; Gardner, 1947a). Other species among rodents as the guinea pig and Octodon degu behave differently. In the guinea pig there is some increase when estrogens are administered for a long time. But quantities many times greater than those with which the anterior lobe increased in the rat up to 7 times in 3 months, produced but a slight increase of the anterior lobe in the *guinea pig* even when administered during a year or longer. Only very exceptionally a considerable increase may be observed in the guinea pig comparable to what is the rule in the rat. In the *degu* where the normal hypophysial weight is of 7 to 10 mg, an increase was produced in 8 months only in 1 out of 29 animals to which stilbestrol was administered by the absorption from subcutaneously or intraabdominally implanted pellets of stilbestrol; the same quantity of stilbestrol administered to 25 rats during 3 to 4 months produced the typical enormous increase of the anterior lobe in *all* the animals (Febres, 1944).

As to *humans* there was, so far I am aware, a single statement of interest in our context. A woman, a desperate case of mammary carcinoma, was given a total of 600 mg of estradiol benzoate during a period of sixty days. A circumscribed hyperplasia of eosinophilic cells, or "adenoma," occupying half of the anterior lobe was found at necropsy (Zondek, 1940). Since estrogen-induced experimental tumors in the rat are chromophobe adenomas one may suppose that proliferation of eosinophilic cells in the related case was not due to the estrogen administered.

A pigmented adenoma of the *intermediate* lobe induced by estrogen in the rat also has been reported (McEuen, Selye and Collip, 1939). Of considerable interest are the findings of Vasquez-Lopez (1944) in the *hamster*. In this species the adenomatous growth of the intermediate lobe invaded both the posterior and anterior lobe whereas the latter did not grow.

D. ATYPICAL EPITHELIAL PROLIFERATION IN THE MAMMARY GLAND

Another striking example of differential response towards estrogens according to the species is offered by the mammary gland. Since the pioneer work of Lacassagne, adenocarcinoma of the mammary gland was elicited by many authorities in different strains of *mice* subjected to estrogens for

a sufficient length of time (see summary in Allen et al., 1939; Lacassagne, 1939; Burrows and Horning, 1947).

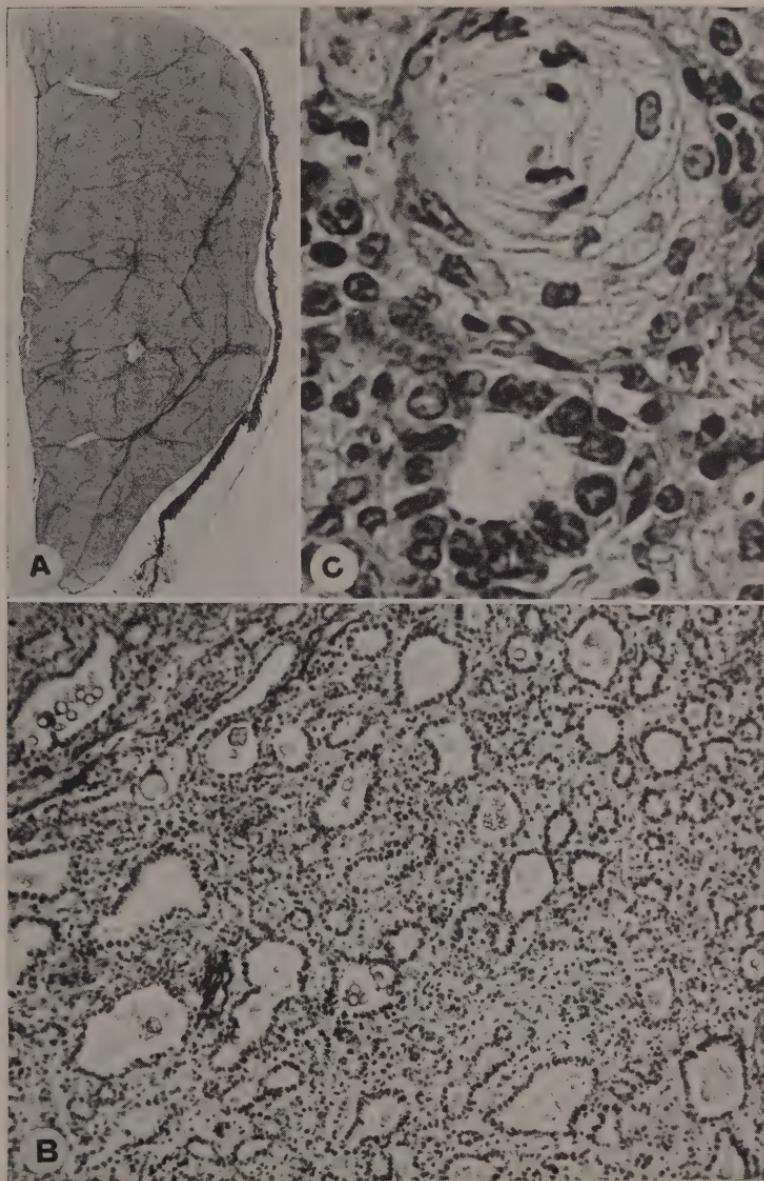


FIG. 50. Fibroadenoma of mammary gland in female guinea pig. 83 inj. of 40 μg of estrad. (benz.), 7 months (I.15). A. Sagittal section. $\times 3.8$. B. Enlarged acini separated from one another by fibrous tissue. Various acini contain droplets. $\times 112$. C. Metaplastic acinus, and acini of normal aspect. $\times 800$.

The behavior of the mammary gland in the *rat* is under similar experimental conditions seemingly different from that in mice but cancer has also been reported in the rat since the well known work of Geschickter (1939, 1940; see also summary Noble et al., 1940; Noble and Collip, 1941a; Nelson, 1944). There are indeed considerable strain differences also in the rat (Dunning et al., 1947).

In the *guinea pig* we have never found adenocarcinoma of the mammary gland. Metaplastic atypical epithelial proliferation is also extremely rare in the guinea pig. It has been reported by Florentin and Binder (1929). In the course of our work the exceptional condition of the mammary gland attracted attention in 3 cases only. In one case (Iglesias; fig. 50) the gland was very hard. In another case the gland protruded in a more pronounced manner than is the rule. Microscopic examination in these two cases revealed that there was no adenocarcinoma but adenofibroma (fig. 50B). There were metaplastic changes in several acini (fig. 50C); but the number of similar acini was a limited one. In a third case (Riesco) a hard nodule was found in the middle of the hyperplastic gland; its structure was coincident with that in the former two cases.

In male and female *cebus* monkeys we were rather astonished not to see any macroscopic response of the nipples or mammary glands during a prolonged treatment with estrogens.

On the contrary, hyperplasia of the breast takes place in *humans* of both sexes when estrogens have been administered for several months (J. A. M. A., 1949). Scarff and Smith (1942) and Fitzsimons (1944) have described "proliferative mastitis" and "gynecomastia" in male stilbestrol workers. Auchincloss and Haagensen (1940) refer to "cancer" induced by estrogen. For more recent references see Exc. Med., Endocrinol.

E. ATYPICAL EPITHELIAL PROLIFERATION IN THE GENITAL TRACT

A highly interesting aspect of differential responses to the prolonged administration of estrogen according to the species is offered by the endometrium. We shall refer only to those forms of atypical growth which are of immediate interest from a comparative point of view; other atypical growths shall be discussed in chapters 8 and 9.

It is well known from the extensive work of various authorities (see summary of Allen et al., 1939) that in *mice* and *rats* the endometrium undergoes under the influence of a prolonged treatment with estrogens epidermization: there is a true squamous epithelium with cornification. Sometimes the whole mucosa undergoes the metaplastic change, i.e. epidermization (fig. 51B). In other cases metaplastic islets may be found scattered among, and surrounded by, hyperplastic cylindric endometrium (fig. 51A). A large epidermoid epithelioma of the uterine horn has been described in a mouse

belonging to a strain resistant to spontaneous neoplasms and receiving estrogen (Lacassagne, 1936). The epithelioma occupied the chorion between the uterine cavity till down to the muscle layer. On the other hand, branching uterine glands may penetrate deeply between the muscle layers;

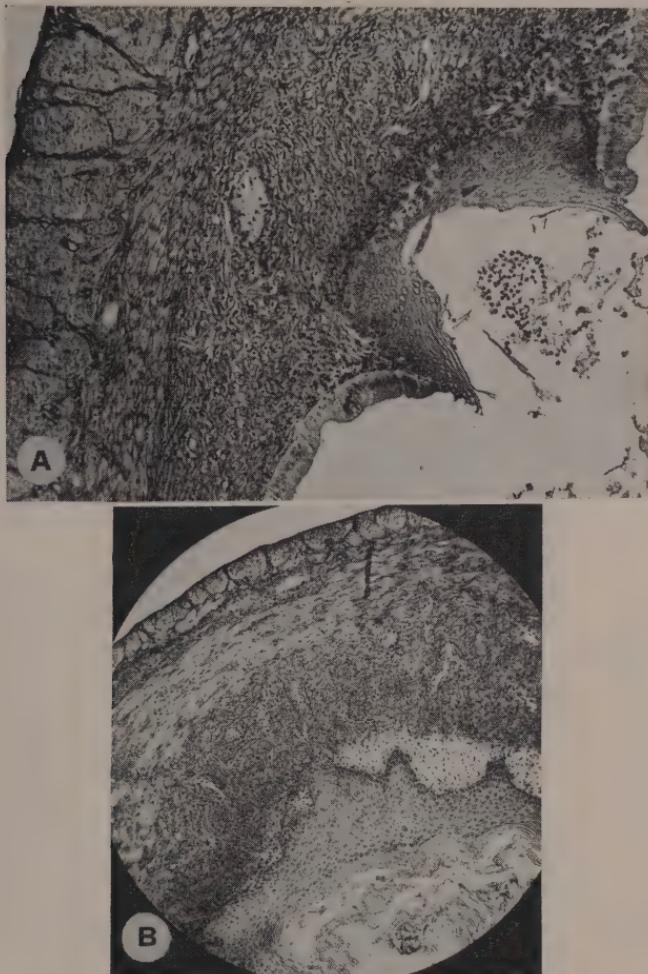


FIG. 51. Uterus of the rat. A. Insular metaplasia surrounded by hypertrophied cells of the endometrium. 42 inj. of 10 μ g of estrad. (17-capryl.), 91 days. $\times 90$. (XIV.R.4). B. Complete epidermization of uterine mucosa in the rat. 38 inj. of 80 μ g of estrad. (17-capryl.), 94 days. (XIV.R.12). $\times 45$.

a condition of "endometriosis" is produced (Lacassagne, 1935b). Branching glands may sometimes produce in mice a conglomeration in the subperitoneal layers, and the serosa may be perforated by these glands (Loeb, Burns and

Moskop, 1936; Loeb, Suntzeff and Burns, 1938).* In one case the gland-like strands breaking through the serosa were entering striated muscle and pushing their way into lymph vessels; the authorities referred in this case to a "cancerous" condition (Loeb et al., 1936). Deep penetration of glands reaching the serosa has been described also in non-injected mice but subject to an experimental hormonal imbalance with prolonged estrous (Pfeiffer, 1939; see also Part III, ch. 18).

In the *rabbit* the branching glands also reach and penetrate the muscle layers. The condition has been referred to as "adenofibromyoma" (Lacassagne, 1935a). The glands may reach and perforate the serosa (Pierson, 1937, 1940; Hofbauer, 1939).

In the *guinea pig* work on estrogen-induced atypical growth has been in former years rather scarce. I know only that of Nelson (1937, 1939), of Dessau (1937, 1938) and several others quoted in ch. 2, A. We were inter-



FIG. 52A

ested, in our research with guinea pigs, predominantly in the action of small quantities of estrogen so as to work under conditions as similar as possible to physio-pathological ones. Metaplasia takes place even when small quantities are administered for a sufficient length of time. Limited areas of the endometrium, sometimes small islets only, become mucified. In the guinea pig metaplastic islands are common (fig. 52A); solid epithelial cords originating from the endometrium or from the glands may also penetrate deeply into the submucosa (fig. 52C; figs. 61 and 62). Stratification becomes sometimes very extensive, especially in experiments lasting many months. But contrary to what is so easily obtained in mice and rats, epidermization of the endometrium has not been noted in our work with guinea pigs, even when the experiment continued for a year or more, or when considerable quantities of estrogens were administered (fig. 52B). Epidermization occurs in the guinea pig subject to the prolonged action of estrogens readily in the cervix. In the normal guinea pig there is a graded sensitivity of the different parts of the genital tract to estrogen (Loeb,

* Also new work in mice of Pan and Gardner (1948).

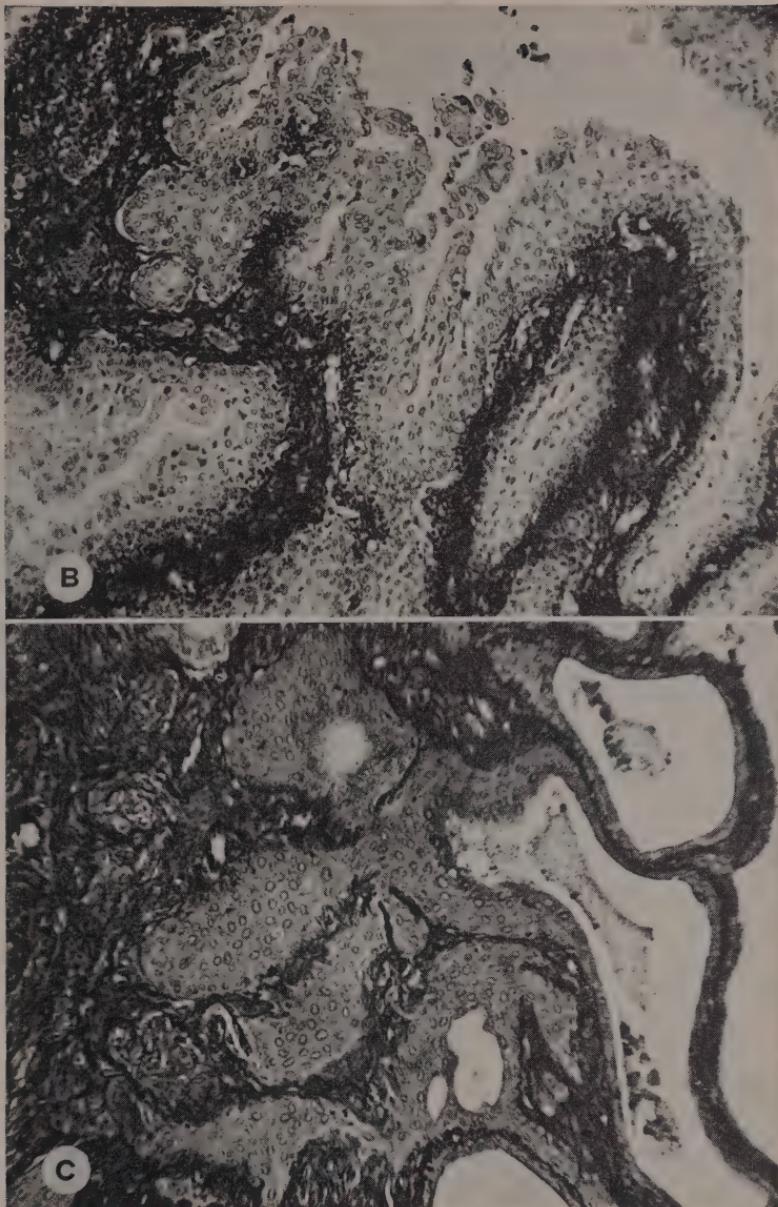


FIG. 52. Stratification of the endometrium in the guinea pig. A. 84 days: 37 inj. of $1 \mu\text{g}$ of estrad. (benz.). Insular metaplasia. Mucified cells on the surface (VI.3). B. 310 days: 129 inj. of $20 \mu\text{g}$ of estrad. (benz.). With the longer duration of the experiment the whole endometrium became stratified but there was no cornification. $\times 112$. (I.22). Compare to fig. 51. C. Cystic glandular hyperplasia. Epithelial cords originating from glands and filling the submucosa. Subcut. pellet, 5 per cent of estrad. Same animal as fig. 34; 244 days. $\times 98$.

1928). In estrus an epidermization in the common cervix takes place, with the mucosa undergoing a change identical to that of the vagina; on the contrary, the mucosa of the individual cervix of the horn is in estrus lined with a mucified epithelium. When the action of estrogen is prolonged, epidermization may extend as high as the individual cervix. But only very exceptionally have we seen a similar change, in the endometrium.** On the other hand, there was in the guinea pig an abundant glandular proliferation, of such a degree we have never seen in the rat. The uterine cavity of the guinea pig may be filled with, or distended by, papillomatous masses of an adenomatous aspect (fig. 54B). These polyps often descend also into the vagina and may even appear outside the genital opening (fig. 53). In

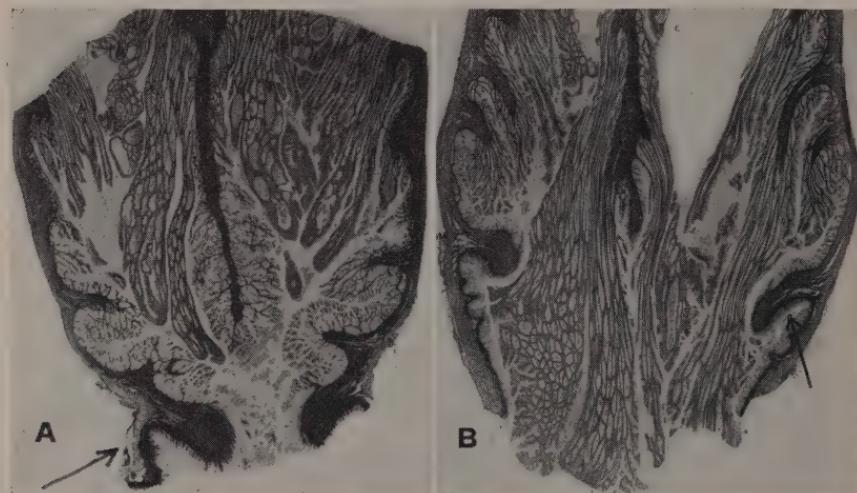


FIG. 53. Adenomatous uterine polyps in guinea pigs, induced with small quantities of estrad. (diprop.). 38 inj. of 10 μg , 87 days. $\times 5$. A. On the left side the polyps approach or enter the common cervix. (VI.34). B. Large polyps have reached the vagina and left the genital opening (prolapse!). (VI.32).

mice and rats where cystic glandular hyperplasia has so often been described no descending adenomatous polyps are mentioned.

We have referred to the fact that metaplasia of the endometrium and so also considerable development of adenomatous polyps can be elicited in the guinea pig already with small quantities of the estrogen. The quantities may be really minute, say a total of 30 to 40 μg given in the course of three months, but under the condition that the estrogen may act *continuously*. That is, when administered through injection of slowly absorbed esterified estradiol (Bellolio, 1939; Lipschutz, Vargas, Jedlicky and Bellolio, 1940)

** For exceptions see ch. 8, and ch. 4, B (observations of Koref and Lipschutz).

(fig. 54B), of stilbestrol or its propionate (Bruzzone, 1942), or through absorption from subcutaneously implanted pellets of the free hormone

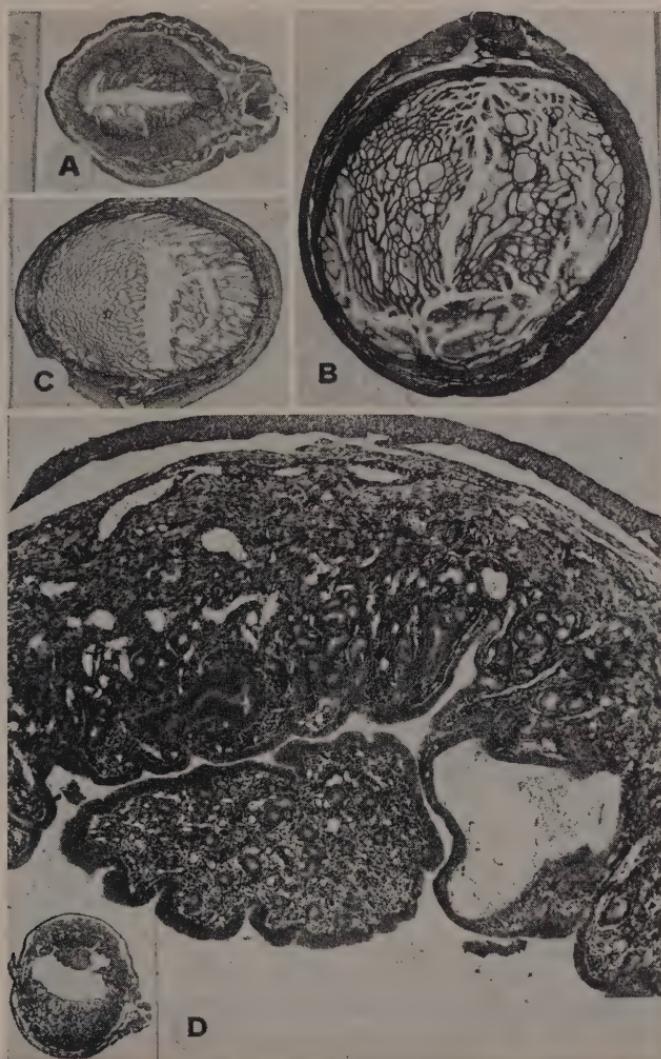


FIG. 54. Comparative action of free and esterified estrogen on the endometrium of g. pig. A. 40 inj. of 20 µg of free estrad., 96 days. Almost normal aspect. Some small glandular cysts are present. $\times 5$. (V.12). B. 38 inj. of 10 µg of esterified estrad. (benz.), 87 days. Adenomatous polyps fill and distend the uterine horn. $\times 5$. (VI.31). C. Early appearance of adenomatous structure with the continuous action of free estrogen. Subcut. pellet of estriol, 21 days, $\times 5$. (XVI.6). See also figure 26. D. Intrauterine polyp of animal with subcut. pellet containing but 1 per cent of estrad. 49 days. $\times 45$. Some glandular cysts. The whole uterus at $\times 5$ in the left corner. (CXXXIV.94).

(fig. 54C). Even when the pellet contains but 1 per cent of the hormone mixed with cholesterol, cystic glandular hyperplasia including polyps may be readily elicited with similar pellets when allowed to act for a time so short as 6 weeks (Barahona, 1949). Mention has already been made of the

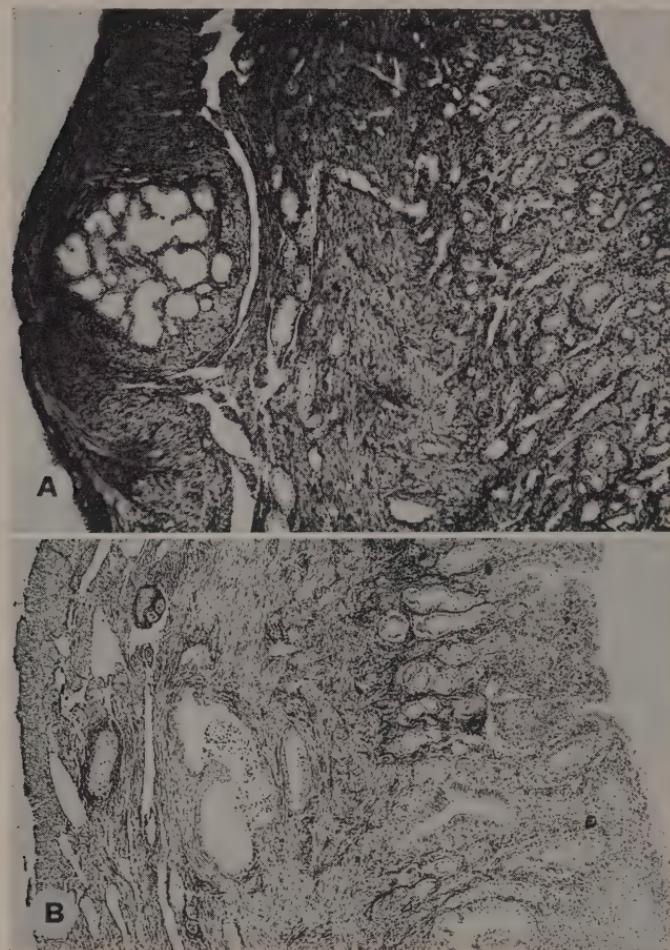


FIG. 55A AND B

fundamental fact that the quantities by which metaplasia and glandular proliferation are elicited in the genital tract of the guinea pig are still insufficient to produce abdominal fibroids even when the experiments are prolonged for several months (see ch. 3, B).

Similar to what has been known since 1935 in mice and the rabbit, penetration of proliferating glands into the myometrium takes place also in the

guinea pig. The phenomenon was first seen in guinea pigs with an hormonal imbalance due to "ovarian fragmentation" with the prolonged action of the animal's own estrogens (Lipschutz, 1936a; fig. 92, 93). In animals receiving estrogens adenomatous, or fibroadenomatous, nodules may be found deeply in the myometrium and reaching the serosa (Lipschutz, 1941; Lipschutz and Vargas, 1941e; fig. 55A). Glands may reach or pen-



FIG. 55. Early invasion of proliferating glands into the myometrium. A. Adenomatous nodule in the myometrium. 46 inj. of 5 μ g stilbestrol, 111 days. Uterine weight —1.5 g. \times 45. (G.p. II St.19). B. Enlarged gland in the superficial circular muscular layers. 37 inj. of 1 μ g of estrad. (benz.). 84 days. Uter. weight 2.3 g. \times 45. (VI.1). C. Gland migrating to the circular muscular layers. 41 inj. of 10 μ g of stilbestrol, 101 days. Uter. weight 2.9 g. No fibroids. \times 90. (IISt.15).

etrate into the myometrium as early as 75 to 85 days after the action of estrogen begins (fig. 55B). Indeed, experiments in mice, rabbits and guinea pigs, in which deep penetration of proliferating glands has been discovered, were mostly of long duration (mice—24 mo., Loeb et al., 1936; rabbits—13 and 28 mo., Lacassagne, 1935a; Pierson, 1940; guinea pigs—33 mo., Lipschutz, 1936a, and 18 mo., Riesco, 1947). But the fact that the glands when under the continuous influence of estrogen may acquire so soon the abnormal faculty of invading the myometrium, is likely to show

that the break from normal to pathological is rather precocious. The longer the experiment lasts the greater is the chance that such a break may take place, and the greater will be the number of glands undergoing this fundamental change; the deeper also will be penetration and the nearer will the

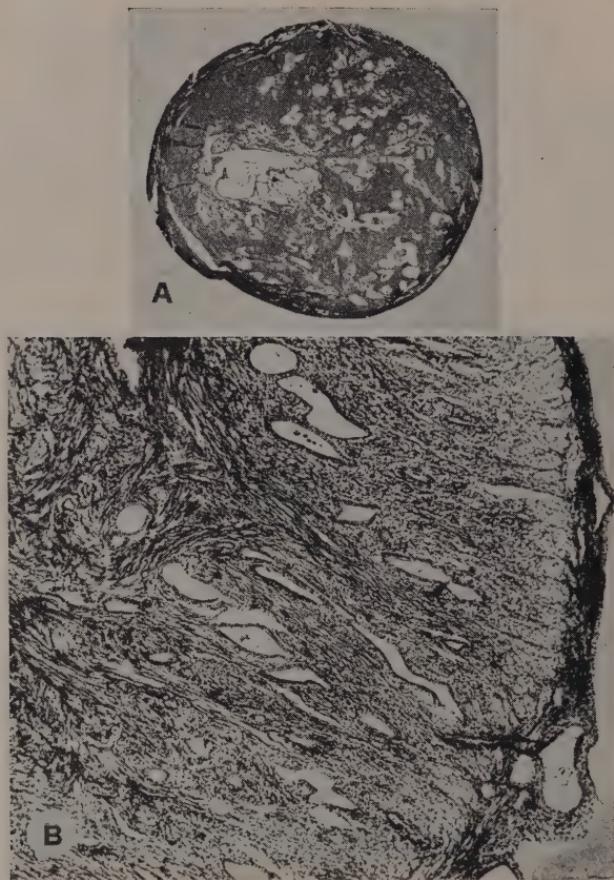


FIG. 56. Uterine glands invading the myometrium. Minute quantities of estrad. absorbed from subcut. pellet containing 3 per cent of the hormone. A. Great number of proliferated glands, partly cystic. 777 days. $\times 5$. (CXXIII.18). B. Glands reaching the serosa. 627 days. $\times 45$. (CXXIII.12).

glands come to the serosa to perforate it. All this explains the great incidence of cases with deep invasion found in new work at 18 months (Riesco, 1946, 1947) and especially at 25 months (unpublished experiments in collab. with Riesco, with the technical assistance of Mrs. Julia Peña; fig. 56, 57). The striking result obtained in this phase of our work may have been due to *small quantities* of estrogen having been administered; the

quantity of the free hormone absorbed per day from a pellet containing but 1 per cent of the hormone is forcibly less than 0.9 μg , according to the

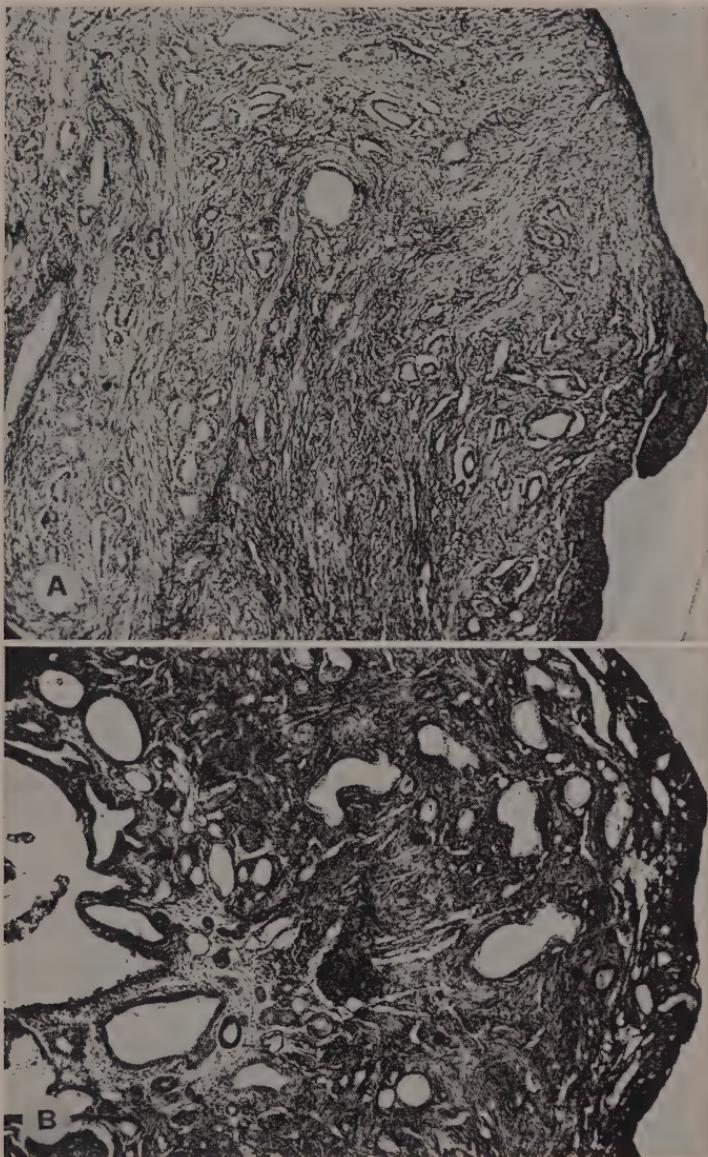


FIG. 57. Uterine glands invading the myometrium. Minute quantities of estrad. absorbed from subcut. pellet containing but 1 per cent of the hormone. A. 435 days. $\times 45$. (CXXIII.28). B. Glands reaching the external longitudinal layer. 870 days. $\times 45$. (CXXIII.3).

diminished surface from which the hormone is absorbed. We shall return to these questions in chapter 9, D, 3.

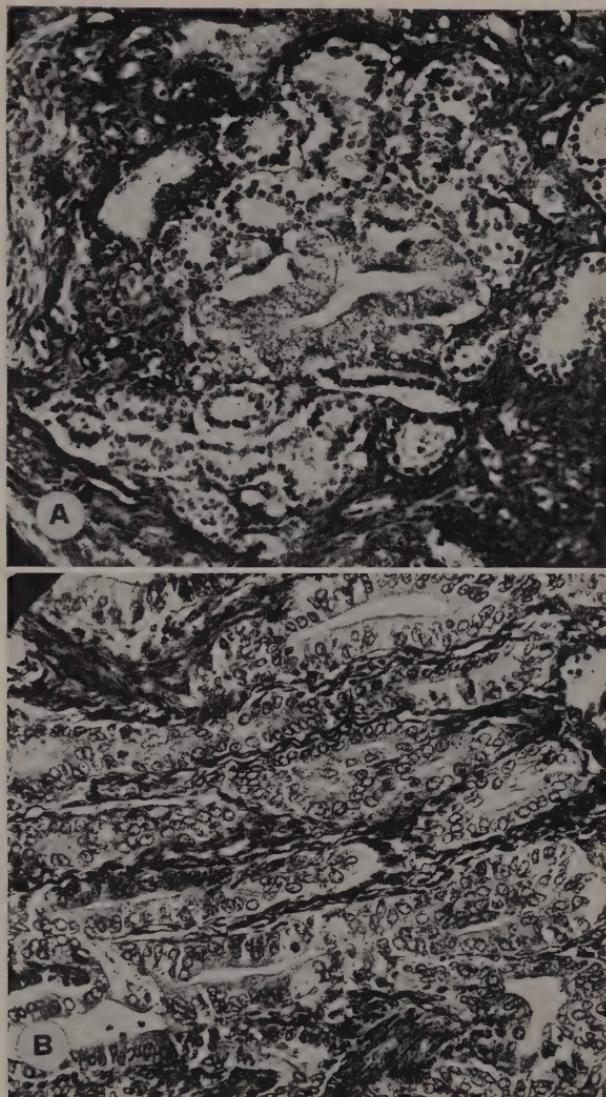


FIG. 58. Different types of glandular structures proliferated under the influence of minute quantities of estrogen. A. Proliferated glands deep in the submucosa. Two types of glands, reminiscent of a picture described in the woman as "endometrium showing adenocarcinoma and adjacent hyperplasia" (see Speert 1948, fig. 1). Pellet, 5 per cent of estrad. 493 days. $\times 200$. (CXXIII.51). B. Crowded proliferated glands in the submucosa. Pellet, 3 per cent of estrad. 867 days. $\times 200$. (CXXXIII.14).

The histologic picture in the guinea pig has been claimed by various pathologists to be similar to that of adenocarcinoma in women whereas others insisted on its being "adenomyosis" or "endometriosis". The break from the normal "non-invasive" to the "invasive" condition of the glands is by no means necessarily a break from normal to "cancerous". This is evidenced by the very existence of non-cancerous adenomyosis* in women, and no less by the fact that in endometrial carcinoma in women "specimens are not unusual in which much or the whole of the endometrium is replaced by a superficial layer of carcinoma with little penetration into the muscle" (according to Welsh, quoted from Willis, 1948, p. 536; Hertig et al., 1949).

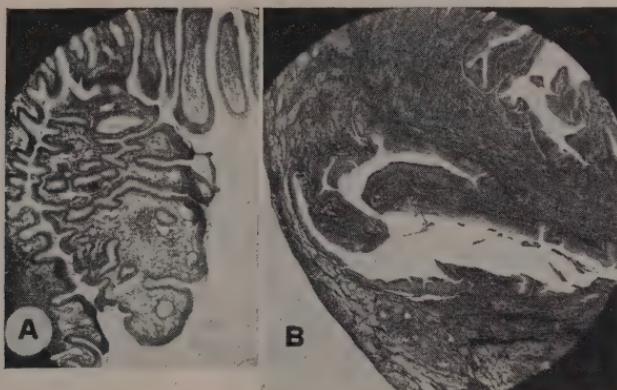


FIG. 59. Glandular proliferation in the uterus of *Octodon degu*. A. Papillomatous masses fill part of uterine cavity. 16 µg of stilbestrol, subcut. pellet 220 days. $\times 5$. (LXVI.26). B. Cystic enlargement of uterine glands pressing against myometrium and coming very near the serosa, and distending but not invading the myometrium. 6 µg of stilbestrol per day, pellet in the abdominal cavity, 168 days. $\times 5$. (LXVI.57).

So far we have not sufficient knowledge so as to decide whether the picture of glandular invasion induced in guinea pigs in our work with minute quantities of estrogen is wholly coincidental with that obtained in mice, and whether it can be termed adenocarcinoma (fig. 58).

Another type of behavior of the endometrium we found in *octodon degu*. In this rodent no metaplasia occurred even when the experiments were continued for as long as 8 months. There was no mucification, no stratification and no epidermization. The cells of the endometrium were hyperplastic; the glands became more numerous; the uterine cavity was filled

* As to adenomyosis, or endometriosis, in women see Goodall (1944) and Willis (1948, p. 535). Javert (1949) points very well also to our experimental problem when writing that "benign endometrial cells are capable of dissemination and metastasis along the same channels followed by endometrial adenocarcinoma."

with, and distended by, papillomatous masses (fig. 59A). The proliferating glands pushed against the myometrium and came near the serosa but with-



FIG. 60. Estrogen-induced changes in the mucosa of the uterine fundus in the South-American monkey *Cebus apella*. A. Glandular cysts filling almost the whole uterine cavity. Different estrogens in the course of 1016 days. $\times 3$. (Mo.6). B. Cystic glands with desquamated cells in the cavity. Stratification of wall of the cysts. Many glands transformed into solid epithelial cords. Two inj. of 200 μg of benzestrol diprop. per week, 513 days (total 31.4 mg). $\times 70$. (XCI.5).

out invading the myometrium, that is to say without penetrating between the muscular layers (fig. 59B). Never was metaplasia observed in our work with octodon degu.

Of considerable interest is the question of the comparative behavior of *anthropoids*: there seems to be a differential response of the endometrium in *rhesus* and *cebus*, the two groups of monkeys which so far have been studied experimentally. It is known from the work of different authorities that cystic glandular hyperplasia is induced in *rhesus* by a prolonged treatment with estrogens. But as far as I know, in *rhesus* metaplastic changes have never been seen in the endometrium of the fundus (see summary Zuckerman, 1940; Pfeiffer and Allen, 1948); metaplasia is in *rhesus* confined to the cervix. In *cebus* we found again cystic glandular hyperplasia of the endometrium (fig. 60A) and metaplasia of the cervix; but contrary to the statement with *rhesus* there was in *cebus* metaplasia also of the fundus in 3 out of 9 cases (Iglesias and Lipschutz, 1947; Bruzzone and Awad; Awad, 1946). In one animal to which estrogens were administered for 513 days the glands were found transformed into massive epithelial cords or in a state of stratification (fig. 60B). The nodular, or insular, character of these metaplastic changes was remarkable. Less pronounced metaplasia of the fundus may appear in *cebus* much earlier than that.

In *women* cystic glandular hyperplasia occurring after the prolonged administration of estrogen has been reported as early as 1933 by Kaufmann to be substantiated by the observations of many other workers. It has been recently claimed that cancer of the corpus appeared in various patients after long continued estrogen administration; whereas this condition bore indeed, a considerable resemblance to hyperplasia and metaplasia there were some cases of estrogen-induced hyperplastic endometria which "caused such an uncomfortable suggestion of cancer in the minds of the experienced pathologists . . . that they advised removal of the uterus as a prophylactic measure" (Corseaden and Gusberg, 1947). Similar statements about patients receiving estrogens have been made by other authorities (Fremont-Smith et al. 1946, 1948; Gusberg, 1947; Novak and Rutledge, 1948; Speert, 1948; Stokes, 1948; Clemmesen, 1948).

There was also, in a *cebus*, an adenocarcinoma of the pericardium (Iglesias and Lipschutz, 1947). Since this tumor has occurred in 1 animal only we have supposed that it was spontaneous.

Adenomatous tumors have been induced by stilbestrol in the cortical region of the *kidney* in the hamster (Kirkman and Bacon 1950). Tumoral growth is, most probably, elicited with the administration of estrogen also in the *ovary* itself (Part III, ch. 21, B, 2; p. 222).

Chapter 7.

NEOPLASTIC GROWTH AND THE CONCEPT OF THE MOSAIC OF TERRITORIES

We may now resume the comparative statements about the atypical proliferative responses to estrogen on the part of homologous tissues in different zoological species. To simplify matters we refer only to those statements which have been dealt with in chapter 6.

The scheme of table 2 gives a striking picture of how widely the atypical, or tumoral, response of homologous tissues to estrogens varies according to the species. Each region of the body obeys its own law as to experimental tumoral responses elicited by estrogens. From the fact that a given tissue, say the peritoneal cover of the guinea pig, responds to a prolonged treatment with estrogens with the production of fibroids, nothing can be foreseen about the behavior of other tissues of the same species. And likewise, from the fact that a given tissue, say the anterior lobe of the hypophysis of the mouse or rat, responds to estrogens with considerable increase or atypical proliferation, nothing can be foreseen about the behavior of other tissues in these species. Reference has also been made to the graded intensity of the proliferative response to estrogen in the genital tract of the guinea pig under physiological conditions (Loeb, 1928); it is the same under experimental conditions (Lipschutz, 1937a) when, with the prolonged action of estrogen, the proliferative response becomes metaplastic. The stratified epithelium with cornification of the superficial layers (epidermization) takes place normally in the vaginal mucosa and in the common cervix only (table 3 (b)). When estrogen is administered for a certain time cornification may occur also in the individual cervix of the uterine horn; adenomatous proliferation prevails in the horn itself which can become stratified, but cornification never takes place (table 3 (c)). A similar differential behavior at different levels of the genital tract has been found in ovarian fragmentation (table 3 (d); p. 184). This graded intensity of response is seemingly also responsible, in part, for cancer of the cervix in women being of the squamous type, cancer of the fundus mostly of the adenocarcinomatous type. Burrows has emphasized that there is a graded intensity of metaplastic response to estrogen also in the accessory genital organs in male mice, the epithelium of the coagulating glands being the first to show metaplastic proliferation,

the seminal vesicles being the next to follow and the prostate the last. Recovery after withdrawal of estrogen takes place in the reverse order (Burrows, 1936a).

The organism can thus tentatively be considered, as to experimental tumoral responses, as a *mosaic of territories* (Lipschutz, 1942b). The behavior of each of these territories in response to the morphogenic action of the estrogen which under certain experimental conditions becomes tumorigenic, is a problem *per se* which can be answered only on the basis of experimental observation.

TABLE 2
Atypical proliferative response of homologous tissues to estrogens

Tissues	Mouse and rat	Guinea pig	Wild caviae (Bolivia)	Octodon degu	Rhesus	Cebus apella
Visceral and parietal serosa	No reaction	Abdominal fibroids and pronounced disseminated fibrosis	No re-action	No reaction	Slight fibrosis (?)	Slight proliferative reaction
Anterior lobe of the hypophysis	Great increase; "chromophobe adenoma." Strain-limited	Very rarely a considerable increase	—	Very rarely an increase	—	—
Mammary gland	Strain-limited adenocarcinoma in mice. Sometimes cancerous in rats	Never cancerous, sometimes adenofibroma and metaplasia	—	—	—	—
Endometrium	Squamous epithelium and cornification. Cyst. gland. hyperplasia. Invasive proliferation of glands, especially in mice	Stratification but never cornification*. Mucification. Cyst. gland. hyperplasia. Large adenomatous polyps. Invasive proliferation of glands	—	No metaplasia. Glandular proliferation	Cystic glandular hyperplasia	Cystic glandular hyperplasia. Nodular stratification

* Exceptionally epithelioma; see chapter 8.

The concept of the mosaic of territories applies also to the abdominal serosa as a whole, or to the endometrium as a whole, or to the cervical mucosa as a whole. Each of these tissues consists of homologous cells; but only a few of these will respond to the prolonged action of the morphogenic factor with tumoral or neoplastic growth. "Many are called but few are chosen"! This statement is one of the fundamentals of experimental tumorigenesis.

The concept applies also to spontaneous tumors. It is known, for instance, that hereditary adenocarcinoma of the mammary gland in certain strains of mice is not linked with hereditary tumors in any other region and neither

with a special sensitivity for carcinogenic hydrocarbons. "It is probable that each type of tumor is hereditarily transmitted without regard to other types of tumors" (Loeb, 1937).

The concept of the organism as a mosaic of territories each of which obeys its own law in its response to tumorigenic growth factors, a concept derived from observations on estrogen-induced conjunctive or epithelial atypical, or neoplastic, growth, is to a certain degree coincident with what has been termed "*competence*" in modern embryology. It is important to keep in mind that the concept of a mosaic of territories must not be taken morphologically or statically. When speaking of homologous tissues of different species, or of different tissues of the same species, or of different but homo-

TABLE 3

	Small doses of estrogen*	Normal estrus or corresponding quantity of estrogen†	Prolonged administration of estrogen	Prolonged action of endogenous estrogen in ovarian fragmentation‡
	(a)	(b)	(c)	(d)
1. Vagina.....	Mucification	Epidermization	Epidermization	Epidermization
2. Common cervix.....	—	Epidermization	Epidermization, sometimes epithelioma	Epidermization
3. Individual cervix.....	—	Mucification	Mucification; sometimes epidermization and epithelioma	Mucification; sometimes epidermization and epithelioma
4. Uterine horn.....	—	Cylindric epithelium	Adenomatous proliferation; mucification; invasion of glands into the myometrium; stratification but no cornification§	Adenomatous proliferation; mucification; invasion of glands into the myometrium

* Or with the simultaneous action of progesterone; or pregnancy.

† Loeb (1928), and own observations.

‡ Own observations; see Part III, ch. 18.

§ Exceptionally epithelioma; see chapter 8.

logous cells of the same tissue, as of different "territories" we always refer to nothing else than to a *differential pattern of potential response* due to a state of reactivity. It is thus evident that the whole concept is in its essence a purely dynamical one. "Competence," a term introduced by Waddington, is a "state of reactivity" (Needham, 1942; p. 112). This state of reactivity is time-conditioned, that is to say, it changes in the course of embryonic development.

To be able to react, or to be "competent with respect to several fates is to be in a condition of unstable equilibrium. Whatever provides the stimulus for the realization of one of the fates which the competences represent will usually have the effect of suppressing the remaining competences or degrees of freedom, and so of inaugurating a condition of more stable equilibrium" (Needham). "Competence is a name

for the actual state of the tissue at, and before, the time when the instability is resolved and one or other path of development entered upon" (Waddington). But "some competences exist in the adult tissues, if we may so term the states of reactivity to hormone action, though these effects are generally reversible" (Needham, p. 682).

When applying the term competence to neoplastic growth some special remarks seem necessary. Reference is made by Waddington and Needham to the condition of an "unstable" equilibrium giving way, under the influence of the stimulus, to a condition of "a more stable" equilibrium. Speaking morphologically the transition, in the course of embryonic development, from "non-differentiated" to "differentiated" is meant. Now, in neoplastic growth something quite "unexpected"—from the point of view of normal morphogenesis!—occurs: The "more stable" equilibrium of an already differentiated say endometrial cell has been shaken by some factor which as to timing, quantity or chemical constitution is new, or abnormal, in any case different from those factors which are active in the normal course of events. The "embryonic" state of an "unstable" equilibrium has been reconditioned, in such a way that a new pattern of response, the atypical, tumoral or neoplastic growth can now take place. One may say that it is, to a certain degree, a kind of "backward development," "not foreseen" in the normal morphogenesis of the species. The idea of atypical cellular proliferation being related in some way to an "embryonic" condition thus reenters, and quite logically, into the realm of the concept of neoplastic growth but in a "dynamical" and no more in a morphological or "statical" sense as in bygone times in the well known concept of Cohnheim or Ribbert, for instance. According to the results of ingenious experiments of Rous and Smith (1945; W. E. Smith and Rous, 1945-1948; W. E. Smith, 1947-1950) the cancerous reactivity of embryonic tissues—stomach, lung, ovary, and bile passages—to methylcholanthrene is probably the same as, if not greater than, that of adult tissues. These authorities have produced squamous cell carcinoma in the glandular portion of the fragmented stomach of the mouse embryo, injected into the adult animal together with methylcholanthrene, in the extremely short time of 44 days. The cancerous structures resembled those of adults. These tumors are transplantable (Smith, 1947). Similar experiments have been reported by Greene (1945)*. It has been argued that the rapidity with which epithelial tumors have been induced with methylcholanthrene in embryonic tissues might be due to the greater ease with which contact is established between the carcinogen and

* See also experiments with the subcutaneous transplantation of adult lung with carcinogen in mice (Horning, 1947), and of fetal lung of guinea pigs without carcinogen (Waddell, 1949). Successful intraocular transplantation of human placenta into the rabbit (Gurchot et al. 1947), and of embryonic mouse tissues into mice of other strains and rats (Browning, 1949) also has been reported.

the reacting layers, compared to adult tissues; with *intradermic* injections in adult mice a cutaneous epithelioma was seen, though in one animal only, as early as 29 days after injection (Gottschalk, 1948). This statement is indeed not conclusive.

Tumors of the pulmonary tissue have been obtained in newborn mice by the administration of urethane to the pregnant animal in the latter half of pregnancy; these lung tumors may be found as early as 3 days after birth (Smith and Rous, 1948).

The finding also may be mentioned here that estrogen-induced cancerous lymphoma in mice shows a lower incidence when treatment begins at the age of 4 months instead of 1 month; the aging of the hemopoietic tissues seemingly diminishes their sensibility (Silberberg and Silberberg, 1949).

A differential response of territories may be due in some cases to a differential threshold, as exemplified by the special reactive pattern with small quantities of estrogen in guinea pigs when most of the animals show the tumoral seed on the serosa of the *spleen* and surrounding parts, without nodules on the serosa of the uterus. The state of reactivity of a territory may be dependent upon special metabolic conditions present in this territory and not in others; one may refer to the liver which interferes in the fate of estrogens by inactivating them, and on whose surface subserous fibroids are very rare. As to the differential response of homologous cells of the same tissue exemplified by the nodular or insular metaplastic proliferation of the endometrium, it can be compared with follicular development in response to the gonadotrophic hormones of the hypophysis. Out of a great number of follicles only a given number enter into development. This "given" number—in compliance with the "Law of follicular constancy" (Lipschutz, 1927b)—is certainly determined by the limited quantity of the gonadotrophic hormones as shown by a wealth of experimental work; it can be infringed by the administration of anterior lobe. But the "choice of the few" in the normal ovary¹—or the *one* in the human species—among the many follicles "called" is determined by the developmental condition of the "chosen" follicles, a condition which manifests itself in a certain pattern of response to the gonadotrophic stimuli.

The differential response of an *homologous* territory of different species must not be confounded with the differential response of an *analogous* organ of different species. The prostatic region offers various examples of this kind. The most striking of these is the differential behavior of the *utriculus masculinus* in the guinea pig as described in a previous section, and of the *so called* "*utriculus masculinus*" in the rabbit. In the latter case estrogen

¹ And even then when the number of eggs shed and entering into embryonic development is doubled or tripled, as stated in the well known work of Smith and Engle and others with the administration of anterior lobe.

does not stimulate the proliferation of the epithelium which, on the contrary, is dependent on androgen as a morphogenic stimulus (Deanesly, 1939b; for other examples see Zuckerman, 1940).

We shall refer again to the concept of territories in the Part II when dealing with the antifibromatogenic action of certain steroids, that is with the inhibition of estrogen-induced tumoral growth *in certain territories and not in others.*

Chapter 8.

IRREVERSIBLE ATYPICAL PROLIFERATION AND NEOPLASTIC GROWTH INDUCED BY ESTROGENS

Estrogen-induced atypical cellular proliferation we have referred to is not autonomous; it is reversible. The abdominal fibroid begins to shrink after the withdrawal of estrogen, and so also the proliferated masses of the endometrium. But there may be exceptions as to the latter. A cystic glandular hyperplasia induced in the guinea pig by the animal's own estrogens (Lipschutz, 1938; see fig. 49 of this paper), or by the administration of estrogens (fig. 100), may persist for weeks and probably also months after the withdrawal of estrogen; the cells lining the cysts diminish in size. In castrated animals with 1 per cent estradiol pellets under the skin since two years the vagina which was open for a certain time may close again; the uterus may be found at necropsy almost as small as in a castrate, and the mammary glands undeveloped. Some accident, possibly the formation of a too thick capsule around the pellet, has inhibited the absorption of estrogen, after the latter had acted for so and so many months. But in similar animals proliferated uterine glands lined with small cells (unpublished work in collaboration with Riesco) may be found deep in the myometrium.

Of considerable interest is the following case (Lipschutz, Iglesias and Vargas, 1939). A guinea pig had been injected for eight months with estradiol benzoate. Then administration of estrogen was stopped, and 4 months later the animal was necropsied. An epitheliomatous proliferation has been found in the endometrium of the upper third of the uterine horn (figs. 61 and 62). There was in the same animal a pronounced regression of the fibroids, and likewise an involution of the mammary glands. The genital opening became closed as in castrated animals. All this shows that there was no source of estrogens any more in this animal. But the epithelioma has resisted.

Whereas irreversible estrogen-induced tumoral growth is exceptional in work with guinea pigs, it has been found repeatedly by various authorities in mice. We do not refer, at this place, to all estrogen-induced tumoral growths which are *structurally* more or less similar to known spontaneous malignant neoplasms as reported by many authorities in different species

but especially in mice (Lacassagne, 1935b, 1938; Loeb, Burns and Moskop 1936; Loeb, Suntzeff and Burns 1938; Suntzeff et al., 1938; Pfeiffer, 1939; Gardner, 1947a) and in rats (Del Castillo y Sammartino, 1937; Zondek, 1937; McEuen, 1939; Korenchevsky and Hall, 1940). We like to lay stress only on those estrogen-induced growths which by their *proved irreversibility* betray their cancerous condition. There is first the *cancer of the uterine cervix* induced in mice with rather small quantities of estradiol benzoate (work



FIG. 61. Irreversible epithelial proliferation in the uterus of the guinea pig. 100 inj. of 20 µg of estrad. (benz.) in the course of 245 days. Sacrificed 114 days after last injection (I.14). A. Uterine horn of almost normal aspect; involution after withdrawal of the estrogen. Hyalinized rest of fibroid in the parametrium. $\times 10$. B. Part of the other uterine horn of the same animal. Polyp containing cystic glands and filling the whole uterine cavity. Solid epithelial cords beneath the endometrium. $\times 10$.

of the Yale group; Gardner, 1947a; Pan and Gardner, 1948). This infiltrating and metastasizing tumor is transplantable (Gardner, Allen, et al., 1938; Allen and Gardner, 1941; Gardner, 1947a). The *pituitary adenoma* in mice seemingly persists or continues to grow after administration of estrogen has been discontinued. In the work of Gardner (1947a) the animals still had large tumors when necropsied at 138 days after withdrawal of estrogen. On the contrary, in rats Deanesly (1939a) and Nelson (1944) reported that after discontinuance of estrogen administration the hypophysis regressed

to its original condition. But even in mice the pituitary adenoma is not transplantable in the strict sense of irreversibility or autonomy: the tumor takes only when estrogen is administered to the host (Gardner, 1947a).

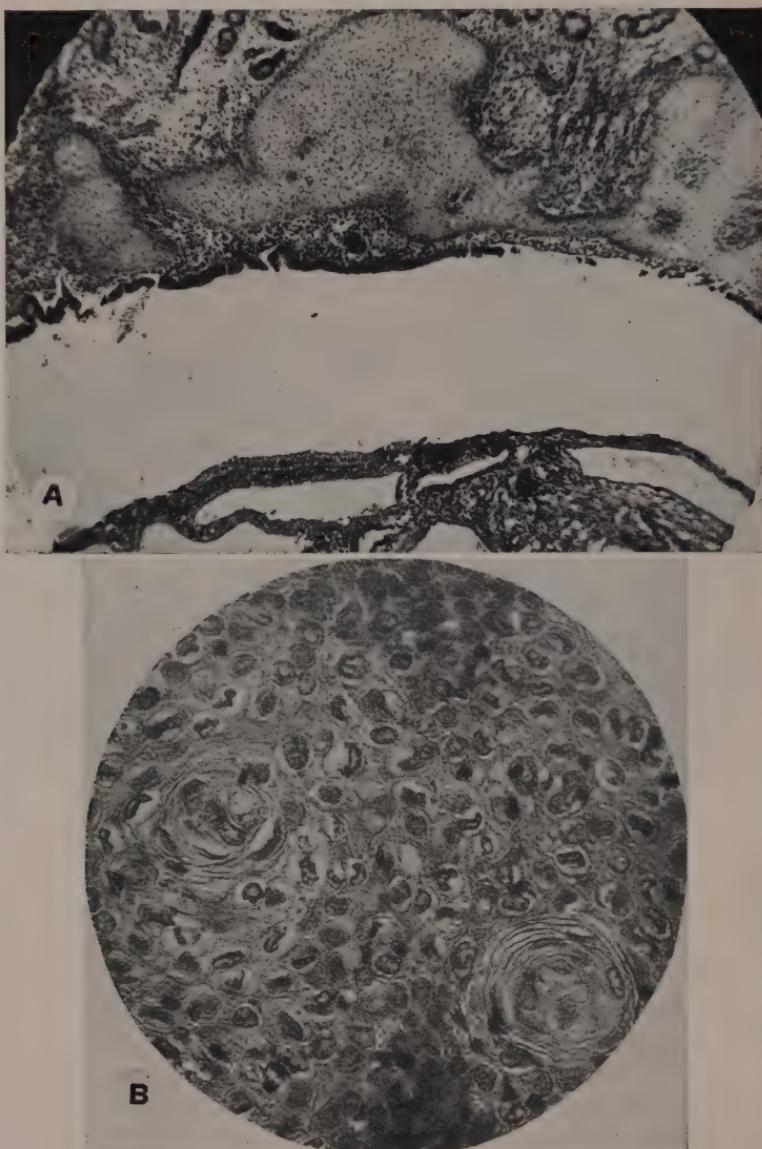


FIG. 62. Same uterine horn as fig. 61B. A. The epithelial cords at higher magnification. $\times 130$. B. Concentric disposition of proliferated cells. Different types of cells. There were also mitosis.

More abundant are the statements about irreversible estrogen-induced neoplastic growth of other tissues. Different workers have elicited *spindle cell sarcoma* in mice by the subcutaneous administration of estrogens. These sarcomata were in general to be found at the site of injection (Cori, 1927; Gardner, Smith et al., 1936; Loeb, Burns, et al., 1937; Burns et al., 1938; Lacassagne, 1937a; summaries Lacassagne, 1939, Cook and Kennaway, 1940). Some doubt may be thrown on their being really due to the action of estrogen and not to that of the lipid solvent (Lacassagne, 1939); but they have been seen also with estrogen injected in aqueous solution (Burns et al., 1938; Burrows and Horning, 1947). Sometimes, though not very frequently, sarcomata appeared also at a distance from the site of injection, as in the vaginal wall (Burns et al., 1938) or in the wall of the urinary bladder (Lacassagne 1937a, 1938). *Lymphosarcoma of the thymus and the lymphatic glands* also was elicited (Lacassagne, 1937b, 1938; Gardner, 1937). Subcutaneous sarcomata and those of the thymus were found to be transplantable (Gardner, 1937; Allen, Hisaw and Gardner, 1939)*. In the guinea pig we have never seen tumors surrounding the subcutaneously implanted pellet of estrogen, free, esterified or artificial, though this way of administration has been used as the routine procedure in our work since more than ten years. Neither were there tumors surrounding intrasplenic and intrahepatic pellets of estrogen. A tumoral thickening of the fibrous capsule enveloping the pellet took place in the abdominal cavity; but this has to be explained by the localizing interplay of the mechanical stimulus as with metallic pellets or glass beads in the abdominal cavity in guinea pigs to which estrogen is administered simultaneously (see chapter 4, B).

* It is also significant that the effect of estrogen in inducing lymphosarcoma in mice is enhanced by X-rays, which by themselves also induce lymphoma in the same strains, and *vice versa* (Kirschbaum et al. 1949).

Chapter 9.

ESTROGENS, NEOPLASM AND CANCER

A. THE DIFFERENT DYNAMICS OF THE TUMORIGENIC ACTIONS OF ESTROGENS

It may be useful to try to sum up actual knowledge on the manifold dynamics of the tumorigenic actions of estrogens:

There is *first* the hereditary mammary adenocarcinoma in mice. It is generally assumed that the estrogen acts only in its capacity of a physiological growth factor, that there is so to say nothing "wrong" or abnormal about the estrogen as to quantitative and timing conditions. The sex rhythm is, in the female of this strain, seemingly a normal one (see summary Allen et al., 1939, p. 572; Gardner, 1941b). There may be indeed certain particularities in the cycle. In a cancerous strain the first cycle presents itself earlier than in non-cancerous ones (Deränger et al., 1945); there may be also a greater frequency of atypical cycles, and they last longer (Armstrong, 1948). There is also the fact known since the work of Loeb that in certain cancerous strains the incidence of mammary adenocarcinoma is much superior in females which have bred than in virgin ones. The figures given more lately by Suntzeff et al. (1941) are convincing: as much as 60 and 43 per cent with mammary tumors in breeding females of the C3H and A strains, as against 19 and 3 per cent in virgins of the same strains, to take only 2 out of 6 of the strains studied. The difference in incidence of mammary tumors is still considerable when comparing females with a different number of litters bred, as between 13 per cent with 1 litter only, and 59 with 5 litters born (work of Jones; see summary of Shimkin, 1945). But none of these statements is liable to suggest a dependence of the mammary tumor in these strains on a *derangement* of the sexual function, or a hormonal imbalance, though there is no mammary carcinoma in these strains without the interference of the estrogenic hormone.

On the other hand, the Korteweg group reported that incidence of mammary cancer may be related to the quantity of estrogen produced by the high-cancer strain without there being a derangement of the cycle when comparing to low-cancer strains. For producing estrus in spayed females of the high-cancer strain *dba* three times as much estrone was necessary than in those of the low-cancer strains (Van Gulik and Korteweg 1940, with

references to the former work of the Korteweg group; Korteweg 1948). A similar statement was made by Shimkin and Andervont (1941) when comparing their high-cancer strain C3H to the low-cancer strain C57. Whereas the vaginal mucosa is in the high-cancer strain so much less sensitive to estrogen, the mammary gland has been shown to be as sensitive as is the

TABLE 4
Different aspects of the tumorigenic action of estrogens

THE ESTROGEN MAY ACT:	CLASSIFI- CATION OF THE ESTROGENIC ACTION	CLASSIFICATION OF THE REACTIVITY OF THE TERRITORY	EXAMPLES
1. As a physiological morphogenic or growth factor, by <i>rhythmic</i> action.	? see text	hereditary abnormal	Mammary adenocarcinoma in mice.
2. As a tumorigenic factor, by <i>continuous</i> action.	abnormal timing	seemingly normal	Reversible atypical growth: experimental fibroids in guinea pigs; uterine adenomatous polyps.
3. As a tumorigenic factor, by <i>continuous</i> action.	abnormal timing	hereditary abnormal, or rendered abnormal by the estrogen	Cancer of uterine cervix in mice; irreversible "epithelioma" of the uterine horn in the guinea pig; lymphoid tumors in mice.
4. Overthrowing the steroid balance by continuous action.	abnormal timing	?	See Part III, chapter 18 and 21.
5. Favoring by continuous action the production of an abnormal tumorigenic substance (steroid?).	abnormal timing	?	?

gland in a low-cancer strain; this is why Korteweg (1948) comes to the conclusion that probably "at least part of the genetically determined disposition to mammary cancer in certain strains of mice is caused by an overproduction of this hormone." Indeed, the quantity of estrogen necessary to bring about the mammary tumor is very small when compared to those generally used in experiments with injections. This has been shown by

Shimkin and Wyman (1946) by the implantation of pellets containing but 2 per cent of estradiol and 98 per cent of cholesterol; 35 per cent of the males developed tumors which do not occur spontaneously in males. But more recently Huseby and Bittner (1948) concluded, on the basis of experimental work, that "the hormonal constitution favorable to the development of breast cancer in virgin female mice is an inherited characteristic."

Whereas the results referring to the comparative hormone production in high- and low-cancer strains may seem conflicting, there is full certainty that the abnormal response of the territory to estrogen depends on the concomitancy of the *hereditary reactivity of the mammary territory* and the *milk factor* as known since the remarkable discoveries of Little, Korteweg and others on the first, of Bittner and many others on the second (summaries Heston, 1945, and other articles in the same volume; Bittner, 1947, with many references to his own work; Bittner, 1948; Dmochowski, 1948, 1949; Heston et al., 1949; Andermont, 1949; Maisin, 1948, p. 71-86, and 1949, p. 125-133; Burrows, 1949, p. 416-442; Pullinger, 1949). Recent experimental statements have suggested that the milk agent may act through the control of the hormone metabolism (Bittner, 1948; Ranadive and Khanolkar, 1948).

The fundamental statements referring to the hereditary mammary tumor, in mice and its dependence on an ovarian hormone have acquired immediate interest since it became known that also in prostatic cancer in men hormonal, i.e. androgenic, action—seemingly without any derangement of the latter—is indispensable for the tumor's ability to grow, in any case in the beginning. We shall return to this question in Section B.

Quite different is a *second* aspect of the tumorigenic action of estrogen: certain experimental tumors appear only when the hormone is allowed to act no more rhythmically but *continuously* as is the case with estrogen-induced abdominal fibroids, with squamous metaplasia of the endometrium, with adenomatous polyps, with hypophysial tumors or with the utricular fibromyoepithelioma. Here atypical cellular proliferation and differentiation in territories more or less limited is induced by the estrogen on account of the special timing conditions under which it is acting on these territories which in themselves are normal. It is only with abnormal timing that the estrogen becomes to play, in these cases, the part of a tumorigenic factor—or a factor of atypical growth, if one does not like to give the name of tumor or neoplasm to these experimental growths by reason of their being *reversible*.

A *third* aspect of tumorigenic action of estrogens is offered when *irreversible* atypical growth is induced by the prolonged administration of estrogen. The hormone acting for a sufficient length of time continuously instead of rhythmically may change the territory, in such a way as to induce an

hereditary change in the respective somatic cells which leads to autonomous or true neoplastic cancerous growth in absence of the primary stimulus which has originally evoked the growth (see also p. 116). The large infiltrating and metastasizing cervical uterine carcinoma as induced by the Yale group with the prolonged administration of estrogen in mice may be especially mentioned here. Mice of no less than 6 different strains which tolerated the treatment for a sufficiently long time, showed this tumor. In experiments lasting more than one year the incidence became very considerable. In a group of 206 females subject to the prolonged influence of estrogen with an average survival of no less than 6 months there were 52 with cervical growth (see Gardner, 1947a; table 1). The spontaneous endometrial carcinoma in the rabbit also must be mentioned here. This malignant growth whose incidence in older animals is apparently considerable, has been known for almost fifty years (references Burrows, 1940) but has been studied especially by Greene and his associates (Greene, 1941a) and by Burrows (1940). The growth invades the muscular wall and produces metastases in many organs. But the primary tumor which is multiple and may affect both horns is concomitant with polypoid growth and cystic glandular hyperplasia of the endometrium, sometimes also with cystic mastopathy and mammary growth of multicentric origin, probably malignant. All this is decidedly in favor of the assumption that this spontaneous cancerous growth of the uterus is dependent on estrogen (Burrows and Horning, 1947).

There is still another type of tumor dependent on estrogen, and certainly a malignant one: the *lymphoid tumor* in mice. We have already referred to the work of Lacassagne with the induction of lymphoid neoplasms by administration of estrogens (Chapter 8). Lymphoid tumors occurring spontaneously in certain strains of mice with an incidence of 75 and even 90 per cent have been described by many workers, and their dependence on estrogen has been studied (Furth, MacDowell, Murphy, Gardner et al.; summaries Furth, 1946; Burrows and Horning, 1947; Gardner, 1947a). The figures of Cole and Furth (1941) referring to the differences of incidence in males and females, and those of the Yale group on the influence of estrogen on the incidence, are striking. In 7 different strains in which the percentage of spontaneous lymphoid tumors was of 0 to 5 per cent the incidence of tumors rose with the administration of estrogen up to 15.4 per cent (Gardner, Dougherty and Williams, 1944; Gardner, 1947a). Whereas it is fully evident that estrogen is implicated in the increase of the incidence of the lymphoid tumor, it would be difficult to classify this neoplastic growth according to the above scheme.

We must refer finally to a rare sarcomatous neoplasm, the *bone tumors* in mice which have been known since long but more recently have been studied especially by Pybus and Miller (1938a, 1938b, 1940a, 1940b). These tumors

—osteoma, osteosarcoma, etc.—can be found everywhere in the skeleton (1940a). The primary malignant cell is believed to be the osteoblast (1940b). Metastases occur (1940a). There is a very pronounced sex-difference in the incidence of these bone tumors (Pybus and Miller, 1938c, 1940a; Miller and Pybus, 1945): 67 per cent in females at an average age of 16 months, and only 23 per cent in males at an average age of 18 months (Miller and Pybus, 1943). But contrary to expectation the incidence was not increased by the administration of estrogen; some bony nodules were elicited by estrogen but they were structurally not coincident with the spontaneous tumor and they were reversible after the withdrawal of estrogen. The incidence of the spontaneous tumor was the same in castrated and noncastrated females but the average tumor age was increased by castration by as much as 4 months. Thus there is reason to believe that estrogen is one of the various factors on which the bone tumor depends (Miller et al., 1943). But there seems to be a difference in the dynamics of estrogenic action or dependence in lymphoid tumors and in bone tumors on one side, and in hereditary mammary cancer in mice on the other side. In the latter case estrogens are essential or indispensable; in lymphoid and bone tumors estrogen is certainly a stimulating factor but it remains questionable whether the hormone is here *conditio sine qua non* as with the hereditary carcinoma of the breast.

It is but reasonable to ask how far the different aspects of tumorigenic action of estrogens as discussed above may apply to pathology in humans. One may question which of the mentioned aspects of tumorigenic action of estrogens may be in play in uterine fibromyoma in women; in cervical carcinoma; in carcinoma of the fundus; in mammary carcinoma; or in fibroids of the stomach or mesentery, two abdominal localizations coincident with those of estrogen-induced fibroids in the guinea pig. Some of these problems will be dealt with in the following sections. But no satisfactory answer can be given as yet to these fundamental problems of human pathology; nor in many cases of spontaneous tumors in animals as evidenced by the two above mentioned examples—the lymphoid tumors and the bone tumors in mice which do not correspond fully to the scheme as given in table 4. Still less can the answer be a satisfactory one as two other aspects of the tumorigenic action of estrogens have yet to be considered, aspects which, though purely hypothetical, are nevertheless of great and immediate interest.

The fourth aspect refers to the suggestion that an overthrow of the steroid balance in the body might be effected by an abnormal timing of estrogenic action. We shall discuss in the Part II the antitumorigenic actions of gonadal and cortical steroids which certainly play a part in the steroid balance (Part III). Production of these steroids depends on the anterior lobe of the hypophysis, and the gonadotrophic and corticotrophic functions of the

latter can be greatly influenced by estrogens, in any case experimentally. The steroid balance in cancer and the physiological antitumoral autodefense are relevant problems which deserve great attention. We shall deal with the underlying experimental evidences in greater detail in the Part III.

The *fifth* aspect is again a purely hypothetical one—the supposition that abnormal tumorigenic substances, or steroids, originate in the body under those experimental conditions which are established by the continuous administration of estrogens. Various statements about abnormal steroids produced in cancer have accumulated since Burrows, Cook et al. have published in 1937 their paper on an hitherto unknown steroid isolated from the urine of a man with a malignant tumor of the adrenal cortex, excessive amounts of estrogenic hormone also being excreted. We refer especially to the extensive work of Dobriner et al. (1947-1948; Reifenstein et al., 1947; also Lieberman and Dobriner, 1945; recent revues: Dodds, 1949; see also Robinson and Goulden, 1949; and Sprechler, 1949; reviews on the metabolism of estrogens: Jailer, 1949; Heard, 1949; Heard and Saffran, 1949).

The chemical nature and the source of origin of those carcinogenic substances which have been extracted by Shabad et al. from the liver of cancerous and non-cancerous individuals is still unknown (Kleinenberg, Neufach and Shabad, 1940; Shabad, 1945; Des Ligneris, 1940a; Hieger, 1940, 1948; Sannié et al., 1940; Steiner, 1942), as likewise of similar substances extracted from cancerous tissues (Menke, 1942; Steiner, 1942; Steiner et al. 1947; see also Mohs, 1948).

B. AUTONOMY AND IRREVERSIBILITY IN THE DEFINITION OF NEOPLASTIC GROWTH

1. *The classical concept of neoplastic growth, and estrogen-induced experimental growth.*

Neoplastic growth is intimately related to atypical proliferation of cells: neoplastic growth is atypical proliferation—insular, or localized, hyperplastic in some cases, metaplastic in others. This statement, though trivial, is essential for any discussion on neoplastic growth, be it “spontaneous” or experimental. For this very reason one cannot avoid speculating about the implication of the different aspects of estrogen-induced conjunctive and epithelial tumoral growth in fundamental problems of neoplastic growth in general. Indeed, an objection has to be raised which at the first glance seems convincing. When referring to neoplastic growth and to cancer one means something *more* than atypical cellular proliferation and differentiation. Neoplastic growth is, according to the classical terminological concept (chapt. 1), “*autonomous*,” and consequently continuous, illimited, *irreversible*, and often destructive, on account of its being independent—according to the

supposition—of the stimulus by which it has been originally induced. "A tumor is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimuli which worked the change" (Willis, 1948; p. 1). The classical concept includes also the capacity to produce metastases as in cancer and, on experimental lines, to take when grafted on another individual and to persist there in its continuous, illimitated growth. From the point of view of current terminology the estrogen-induced adenomatous polyp or the estrogen-induced fibroid is not neoplastic growth. But from the onset one must be aware that a terminological concept, or definition, is but an approach to, and for this very reason often far from, the reality of things. It may then seem well justified to ask what can we learn about neoplastic growth from what is known on estrogen-induced atypical growth.

Before entering into the discussion of this problem it will be useful to recapitulate in table 5 the different types of tumoral growth which have been produced experimentally, by so many workers, and in very different species, by the administration of estrogens.

Most of these types of hyperplastic or tumoral growth are certainly reversible. In accordance with the definition of neoplastic growth given above the majority of the tumoral conditions of table 5 are not tumors or neoplasms. There is indeed also cancer of the uterine cervix (No. 7) and mammary adenocarcinoma in mice (No. 9). There was exceptionally an irreversible epitheliomatous growth of the endometrium (No. 4); there are also the different true neoplasms of the lymphatic system (No. 21 and 22). But the estrogen-induced irreversible neoplasm is rather exceptional when compared to the reversible condition which seems to be the rule. On the other hand, the limit between both is not a sharp one; reversible growths may exhibit behavior which brings them near to autonomous neoplastic growth—their capacity to invade the tissues (No. 5 and No. 12). Invasion has always been associated with the autonomy of neoplastic growth (see especially the work of Greene and Murphy, 1945). The above statements show that with estrogens both hyperplastic and neoplastic autonomous growth can be elicited but also growth which, apparently, nearly borders the latter. In the classical concept there is no place for these transitional conditions. One may indeed argue and very reasonably, that the classical concept, as derived from observations on spontaneous neoplasms in humans, cannot be shaken for not doing full justice to experimentally induced growths in animals. Let us then take two striking examples from human pathology in which new knowledge has been acquired in recent time: the benign uterine fibromyoma and the malignant prostatic cancer.

2. The classical concept of neoplastic growth, and modern knowledge on certain spontaneous neoplasms in humans

One has tried to adduce evidence that estrogens have an important part in the genesis of uterine fibromyoma in women. It has been claimed that an increase of the uterus is often concomitant with fibroids; that there may be cystic glandular hyperplasia and uterine bleeding. A cystic condition of the ovary betraying an abnormal endocrine ovarian activity with prolonged follicular phases also has been reported in cases of uterine fibromyoma. All

TABLE 5

Hyperplastic and tumoral conditions induced by the administration of estrogens

1. Cystic glandular hyperplasia of the endometrium.
2. Adenomatous polyps of the endometrium, with prolapse.
3. Insular metaplasia of the endometrium.
4. Epithelioma of the endometrium.
5. *Invasion* of proliferated uterine glands into the myometrium.
6. Epithelioma of the uterine cervix.
7. Cancer of uterine cervix: *metastases; transplantable*.
8. Mammary adenofibroma.
9. Mammary adenocarcinoma: *metastases; transplantable*.
10. Uterine fibroid.
11. Uterine fibromyoma.
12. Fibroid of abdominal serosa; *invasive*.
13. Fibroid of thoracic serosa.
14. Hyperplasia of prostatic stroma.
15. Metaplasia of utriculus, urethra, and so on.
16. Utricular fibromyoepithelioma.
17. Fibromyoma of ductus deferens.
18. Interstitial-cell tumor of the testicle*: *metastases, transplantable*.
19. Chromophobe adenoma of the anterior lobe of the hypophysis.
20. Adenoma of the intermediate lobe.
21. Lymphosarcoma of the thymus: *transplantable*.
22. Sarcoma of the lymphatic glands: *metastases*.

* For this type of estrogen-induced growth see Part III.

this would be in favor of uterine fibromyoma being due to some disturbance of the ovarian endocrine cycle (see the pioneer work of Witherspoon, 1933; summaries Lipschutz, 1939a; Burrows and Horning, 1947). All these clinical and pathological findings denounce prolonged estrogenic action; but to make the statement valid from the point of view of etiology it is necessary to show that the symptoms mentioned are *always* present in uterine fibromyoma. According to Witherspoon (1933) and C. Mayer (1948) the cystic condition of the ovary and the glandular hyperplasia of the endometrium are really always, or almost always, concomitant with uterine fibroids; but

other workers have questioned this concomitancy (Taylor, 1947). On the other hand there is the well known fact that uterine fibroids cease to grow when the menopause begins or when castration is performed; there may be even regression. As to this uterine fibromyoma behaves like the estrogen-induced experimental fibroid. The behavioral analogy between both is very significant: there can scarcely be any doubt that fibromyoma in women, like the experimental fibroid, needs estrogen to be able to grow and to survive. Uterine fibromyoma in women is not autonomous and neither is it irreversible as expected according to the classical concept of neoplastic growth. Forty six years ago a then famous German gynecologist wrote emphatically that "The origin of fibromyoma is still surrounded by impenetrable darkness and as to this fibromyoma ungloriously joins the remaining neoplasms" (Döderlein, 1904). Today fibromyoma should be declared not to be a neoplasm at all when judged from the point of view of the classical concept of neoplastic growth.

Let us now see whether the classical concept of neoplastic growth still fully applies to such a malignant tumor as prostatic carcinoma. When discussing this question it may be the best to compare it with an other well known type of carcinoma about whose malignancy there is full unanimity: the hereditary adenocarcinoma in mice. In the absence of two concomitant stimuli, the milk factor and the estrogen, the hereditary neoplastic faculties of the mammary cells lay dormant. But the neoplastic growth when already produced becomes independent of the hormone: the mammary carcinoma is transplantable. What is known about this malignant tumor would thus seem to be in full agreement with the classical concept that neoplastic growth is autonomous and irreversible. As to prostatic carcinoma much new knowledge has accumulated since the outstanding work of Huggins; quite unexpected insight into the dynamics of malignant growth has been produced and new, interesting problems have arisen. As with mammary carcinoma in mice, prostatic carcinoma in men comes into existence only when a sex hormone, the testicular androgen, is present in the body. But contrary to the prevailing data on mammary carcinoma in mice, prostatic malignant growth in men, once produced, remains for a certain time dependent on the hormone: when androgens are withdrawn through castration, or when androgenic action is antagonized through administration of estrogens, further cancerous growth of the prostatic epithelium ceases and tumoral cancerous masses even may disappear. It has recently been claimed that this dependence of prostatic cancerous growth on androgen can be proved experimentally. According to Greene and others (1941-1947), grafts of human cancerous tissues into the anterior chamber of the eye of the guinea pig or mouse are liable to take and develop whereas benign tumors never do (see also section D). A graft of prostatic carcinoma in the eye of

the guinea pig takes and develops only when androgen is present; whereas it takes in noncastrated males it fails to take in castrated ones, or in females, unless considerable doses of testosterone are administered (Hovenanian and Deming, 1948).

With prostatic carcinoma in the human body, things seem later on to change: there is a moment when absence of androgen through castration, or administration of estrogen, is no longer capable of counteracting cancerous growth. These are the cases in which improvement due to castration or to estrogen-treatment is again followed by disease, or the cases which resist any treatment with estrogens from the very beginning (Huggins, 1946, 1947, 1949; Thompson, 1948). But the clinical work done by Huggins and his followers has given certainty about a phase in prostatic cancer in which the malignant condition is still reversible to a certain degree; the malignant growth acquires autonomy only later on. It is likely that there is a similar transitory phase of reversibility, or dependence on estrogen, in mammary carcinoma in mice which in general will escape knowledge on account of being of short duration and difficult to detect. This concept has been substantiated by the work of Foulds (1947). He has reported that mammary tumors originating in hybrids of the cross C57 bl. fem. \times R3 mal. behaved differently when transplanted into females and males, the latent period being longer in the latter or there being no growth at all. The difference was annulled by the administration of estrogen. The dependence on estrogen was sometimes lost after transplantation. Browning (1948) working with spontaneous mammary tumors of the C3H strain and using Greene's method of intraocular transplantation (see p. 110) has found that young tumors did not grow when grafted into animals of other strains. Growth appeared gradually but promptly when later stages of the tumor were grafted.

The indisputable fact that there is in the evolution of prostatic carcinoma a phase in which this malignant metastasizing neoplasm ceases growing and enters into regression when a specific hormone is withdrawn is at variance with the prevailing idea that malignant neoplastic growth is always autonomous and irreversible.

When referring to the role of estrogen in mammary carcinoma in mice, or of androgen in prostatic carcinoma in men, one does not feel inclined to label these steroid hormones as carcinogenic stimuli. The "cause" of mammary carcinoma—if by cause is to be understood the factor, or stimulus, by which a territory is rendered liable to give origin to a neoplasm under otherwise normal conditions of the body—is not the estrogen but the hereditarily transmitted reactivity of the mammary territory (omitting the milk factor so as to avoid complication!). And the "cause" of prostatic carcinoma is not the androgen. But estrogen and androgen being necessary for the evolu-

tion of these neoplasms one may call them specific hormonic factors of "realization" of the potential cancerous response of a territory with an inherited reactivity as in mammary carcinoma, or of a territory which has been changed by some unknown factor as we have to assume in prostatic carcinoma (see "initiation" and "promotion" in section D). Indeed, one may argue that this recourse to genetics, or to a virus, with the purpose of avoiding the hormone as a carcinogenic factor, procures but meager consolation from a *practical* point of view. The cause of mammary carcinoma in breeding females of certain strains, versus the non-cancerous virginal females of the same strain, is breeding, that is to say, an hormonal condition, possibly longer action of estrogen (?). And similarly, from the rather "narrow" but unavoidable point of view of the medical practitioner and the patient, the cause of prostatic carcinoma in its phase of reversibility, in a man with a normal testicle—versus the castrate in whom prostatic cancer is unknown—is certainly the androgen!

C. THE COMPARATIVE TUMORIGENIC ACTION OF ESTROGENS AND HYDROCARBONS

There seems to be a wide gap between estrogens and carcinogenic hydrocarbons: only occasional findings of really autonomous and irreversible neoplastic growths induced through the former, and true neoplastic growth so easily induced through the latter. With the recourse to genetics and to a virus the estrogen seemed to be eliminated as a carcinogenic stimulus also in mammary cancer in mice. Consequently the conditions of the carcinogenic action of hydrocarbons are often believed to be fundamentally different from those of the tumorigenic action of estrogens. But this clear cut division between estrogens and hydrocarbons is a rather dangerous oversimplification (Lipschutz, 1943).

Let us take first the *genetical constitution* whose paramount importance in mammary adenocarcinoma in mice is so well established. Implication of genetics in the carcinogenic action of hydrocarbons is known since long through the work of Andervont, Boyland, Bonser, Lacassagne, Shimkin, Warren a. oth. (summary Cook and Kennaway, 1940, p. 425). It has been made evident especially by the extensive work of Strong and his associates with methylcholanthrene in 15 inbred strains of mice (Strong, 1947; Burdette and Strong, 1943). Susceptibility to induced fibrosarcoma varies from strain to strain; some are highly susceptible, others have high resistance. The genes for high susceptibility are linked with the genes for coat colour (Strong, 1946b). In his selection experiments towards resistance to fibrosarcoma induced with methylcholanthrene, Strong has reduced the percentage of susceptibility from 92 per cent in F_7 to 36 per cent in F_{12} (Strong, 1946a). A mouse of a certain strain develops under the influence of estrogen

a mammary adenocarcinoma because it has a peculiar genetic constitution; in the same way—to use the words of Strong—a mouse develops a fibrosarcoma following the subcutaneous injection of methylcholanthrene because it has a peculiar genetic constitution (Strong, 1946b). In rhesus monkeys a neoplasm never has been produced with methylcholanthrene. "One must conclude, therefore, that for this species methylcholanthrene is not a carcinogen, or at least, if it is, the evidence for such a conclusion is still not available" (Strong, 1949). The susceptibility of the rabbit, rat and especially the guinea pig also is very different from that of mice (summ. Warren and Gates, 1941; Shimkin and Mider, 1941; Shabad, 1945; Mosinger, 1945; Berenblum, 1949; see also Foulds, 1939, with thorium dioxide). With an unilateral recourse to genetics some authorities are inclined to deny the tumorigenic or carcinogenic faculty of estrogen; with a similar procedure it would not be difficult to deny the carcinogenic faculty of methylcholanthrene or other hydrocarbons!

The gap between carcinogenic hydrocarbons and tumorigenic estrogen, the first inducing *autonomous* growth and the other preferably *reversible* growth, is not so unbridgeable as it seems at first glance. There is now sufficient evidence that irreversible and transplantable cancer induced by hydrocarbons is but an evolutional endpoint of those changes which have been elicited in the stimulated tissue. Papillomas of the skin induced in mice by a single painting with methylcholanthrene may regress (Mider and Morton, 1940). Regression of "carcinomatoids" elicited by tarring the ear of rabbits also has been reported; the growth may disappear not only when tarring is stopped but even while continued (Rous and Kidd, 1941). Similar statements have been made in work with tobacco tar in rabbits (Flory, 1941). The term carcinogenic is thus "applied to agents which not infrequently call forth a horde of benign neoplasms for every ultimate one that is malignant" (Rous, 1943a).

Of great interest are here also certain statements about the microscopical evolution of sarcoma elicited in the rat by subcutaneously injected hydrocarbons: at the beginning there is seemingly only granulation tissue (Des Ligneris, 1940b) to become subsequently sarcomatous and transplantable.

After all this it would be a very bold overstatement simply pronouncing that estrogens and hydrocarbons differ by the latter being carcinogenic and the first not. Things are much more complex than that as shall become evident in section D.

D. THE EVOLUTIONAL PHASES OF NEOPLASTIC GROWTH

1. *The evolution of spontaneous and induced cancer*

The statement that true experimental neoplasms including cancer as induced by hydrocarbons may be preceded by a condition of reversibility

has been of interest to us on account of smoothing the contradiction in the behavior of estrogens on one hand, hydrocarbons on the other hand, in tumorigenesis. Transition from hyperplasia to neoplastic growth has been admitted also in human pathology. The most interesting example of such a neoplastic conversion of benign cellular proliferation is probably that of adenocarcinoma which may develop later on in patients with endometrial hyperplasia, there being apparently a gradual transition as to structure from benign hyperplasia to malignant adenocarcinoma. Novak and Yui (1937) were the first to lay stress on this eventuality basing their conclusions on very extensive studies. Hyperplasia is known to be dependent on estrogenic action. The existence of hormonal factors in endometrial carcinoma also has been discussed repeatedly, and more recently by a group of American gynecologists (Woll et al., 1948; Hertig et al., 1949; see also p. 87). The findings of Greene and Burrows in the rabbit with spontaneous endometrial carcinoma also have to be recalled here (see p. 101). We have already referred to prostatic cancer which after a phase of dependency and reversibility may reach autonomy and irreversibility, and to statements about a similar behavior of spontaneous mammary cancer in mice (p. 107). The remarkable findings of Greene and his students (1940-1949) with the transplantation of tumoral tissue into the eye of the guinea pig and mouse would be in favor of the concept that certain spontaneous malignant tumors in the rabbit or the human body attain autonomy only after a certain time of evolution. A striking example out of a number of human cancers may be quoted from the work of Greene and Lund (1944): 2 takes in 90 days out of 14 transplants of the 2nd recurrence of a fibrosarcoma; 7 takes in 21 days out of 9 transplants of the 3rd recurrence of the same tumor which were made 4 months and 10 days later. "Autonomy is the outcome of continued development and is not a common attribute of all neoplastic cells" (Greene and Lund, 1944; see also Greene, 1940).

Other workers have indeed been less successful than Greene in this kind of experiments. Human malignant lymphomas (leukemia, lymphosarcoma, Hodgkin's disease), and canine malignant lymphoma did not grow in the eye of alien species (guinea pigs, rabbits); mouse leukemia grew only in the same strain (Lushbough and Steiner, 1949). Lymph nodes removed from patients with Hodgkin's disease, metastatic cancer of the thyroid, and human lymphosarcoma, did not grow in the eye of rats (Hoffmann and Rottino, 1949). One partial success was obtained with the transplantation of fibrosarcoma and embryoma of the kidney of childhood (Eichwald, 1948). Only 1 out of 40 human malignant tumors, an adeno-acanthoma, survived in the anterior chamber of the eye of 167 guinea pigs (Morris et al., 1950). But on the other hand, considerable success has been reported by various authorities (Freeman and Zimmerman, 1944; Jones et al., 1946; Dyer and Kelly, 1947; Schilling et al., 1949). In the extensive work of Schilling a. oth. growth occurred in 8 out of 36 cases of different malignant tumors of humans; in a case of breast cancer growth occurred in

as many as 6 generations of transplants from eye to eye. The conclusion was reached by these workers that growth of the tumor in the eye of a heterologous host is definite evidence of a high degree of autonomy.¹

The succession of two evolutional phases, those of dependent and seemingly autonomous growth, is real, in any case in some instances both with spontaneous and experimental neoplasms, and the question arises about the dynamics of this succession. One may try to express the succession of the two evolutional phases in the following words: in the phase of dependent growth atypical proliferation and differentiation is going on only in the presence of the stimulus which has induced in the hitherto normal territory neoplastic potentialities; on the contrary, in the phase of autonomous growth proliferation is going on in absence of this stimulus. However, this scheme is not warranted by what is known on the dependence of spontaneous tumors on hormones: prostatic carcinoma is dependent on androgen but the latter is certainly not the stimulus to which the neoplastic potentialities of the prostate are due; mammary cancer in mice is dependent on estrogen but the latter, under normal timing and quantitative conditions, is not responsible of the neoplastic potentialities of the mammary gland. New knowledge on the mechanisms underlying the action of carcinogenic hydrocarbons has likewise substantiated the point of view that proliferation in the phase of dependency can be established by agents different from those able to induce neoplastic potentialities. The work of Rous and his associates was fundamental here (Rous and Kidd, 1941; MacKenzie and Rous, 1941; Rous 1943; Friedewald and Rous, 1944). Papillomatous growth induced by tar and hydrocarbons in the skin of the ear in the rabbit may again disappear, as already insisted upon, especially when treatment has been discontinued. When treatment is resumed cancerous growths appear at places where there were formerly reversible growths or where there was no growth at all. In the second treatment the tar or hydrocarbons can be replaced by agents which themselves are not able to induce cancer, as for instance turpentine. From these observations Rous and his associates have drawn the conclusion that the hydrocarbon when inducing cancer acts in two stages. In the first stage a change is produced in the affected cells by which the latter acquire a new pattern of reactivity, that is to say neoplastic potentialities; it is the stage of "*initiation.*" In the second stage the initiated

¹ We do not refer to the heated discussion around the application of the work of Greene to diagnosis of cancer, as we have no personal experience in this field. Ample work with auto-, homo- and hetero-transplantation of tumors of cold-blooded vertebrates has been done by Lucke and Schlumberger (1949). Kidney carcinoma of the frog becomes as readily established in the eye of alien frog species as in the eye of the natural host. There were good results also with transplantation into the toad. No progressive growth occurred after transplantation into fishes or reptiles.

cells are stimulated by the hydrocarbon, or a replacing agent, to proliferate; it is the stage of "*promotion*" (Friedewald and Rous, 1944). After a certain time of action the cells are enabled to proliferate autonomously, without the promoting agent being necessary any more. In the stage of initiation the cells under the influence of the hydrocarbon are brought into a condition comparable to that of the mammary cells in certain strains of mice in which the milk factor has acted and in which estrogen, a growth factor seemingly normal in every respect, promotes cancerous proliferation. The neoplastic potentialities induced by the hydrocarbons may remain in abeyance or dormant for a time without proliferation taking place; in a similar manner the cells of the mammary glands of castrated females in cancer-strains remain, even in the presence of the milk factor, dormant, or latent, neoplastic cells.

The concept of Rous has been substantiated by the findings of different workers as Berenblum and associates (1941-1949; Shubik, 1950), Mottram (1944), Rusch and associates (1944, 1946, 1948; see also Kline and Rusch 1944; Lavik et al., 1942). A single painting with the carcinogen applied to the skin in mice may be rendered effective in producing benign or cancerous growth, when followed by the application of croton resin which by itself has no carcinogenic activity (Mottram, 1944); the incidence of tumors after a single painting with the carcinogen was not affected when there was an interval of as long as twenty and even forty weeks before the prolonged application of croton oil (Berenblum and Shubik, 1947, 1949).

The same concept has been applied recently also in work with the experimental production of tumors of the thyroid. Multiple adenomata of the thyroid were produced in the rat by 2-acetylaminofluorene (AAF) followed by the administration of goitrogenic, or antithyroid, compounds as allyl-thiourea or methyl-thiouracyl (Bielshowsky, 1945, 1947; Hall, 1948). The carcinogen AAF has been supposed to be the initiating and the goitrogen the promoting agent. Conditions are here, indeed, more complicated than in the above mentioned work with carcinogens acting in the skin. The carcinogen, by itself, does not elicit multiple adenomata; but when the rats received as little as 4 doses of AAF, and 18 weeks later treatment with methyl-thiouracyl was started all animals developed multiple adenomata in the course of the following 13 weeks. It was from these results that the conclusion was reached that the carcinogen transforms normal thyroid cells into neoplastic cells and that the neoplastic potentialities of these cells remain dormant up to the moment when the goitrogenic agent enters into play (Hall, 1948). However, the goitrogenic agent when given alone has been shown to induce in rats even transplantable malignant tumors (Purves and Griesbach, 1946; Bielshowsky et al. 1949); adenomata have been induced in mice (Gorbman, 1947). But it remains true that a small dose of AAF accelerates the appearance of benign adenomata without indeed hastening the development of malignancy (Hall and Bielshowsky, 1949), and without being able, as already stated, to produce by itself the thyroid tumor unless partial thyroidectomy has been performed (Bielshowsky, 1949), or unless goitrogen is given subsequently (Hall, 1948). The goitrogen causes a thyroxine deficiency, and it is remarkable that the transplantable malignant tumors take exclusively in partially or totally thyroidectomized

animals, or in animals receiving methyl-thiouracyl, and as to this there is no difference between carcinogen- and goitrogen-induced tumors (Bielshowsky et al. 1949). These findings are much in favor of those authorities who assumed that the thyroid tumors elicited by the prolonged administration of goitrogens were due to the continued stimulation of thyroid cells by the thyrotrophic hypophysial hormone (Bielshowsky, 1949) without thyroxine being produced. The goitrogen-induced thyroid tumor is seemingly the result of an hormonal imbalance like other tumors to which attention shall be paid in Part III (see also table 6). The question remains open whether the thyrotropic hormone is acting by continued stimulation as an initiating or promoting factor.

It is evident that two entirely distinct mechanisms as initiation and promotion are put into action when a hydrocarbon induces cancer in the skin. As we have seen this conclusion is based on sound experimental evidence. On the contrary, transition from reversible to irreversible proliferation, or acquisition of autonomy, is an event as obscure as before (see Rusch and Kline, 1946). It is very probable that neoplasms vary greatly as to the mechanism underlying this transition. It suffices to remember that characteristics of irreversible malignant growth may be present already in the phase of reversible proliferation of certain growths as metastases in prostatic cancer,* or invasion with estrogen-induced abdominal fibroids. Recently Foulds (1949c) has reached a similar conclusion on the basis of extensive studies on the growth, responsiveness to hormones and transplantability of mammary adenocarcinoma in mice. "*Malignancy*" is not a single character. The typical malignant tumor of the textbooks is the result of proportionate development of all the characters proper to malignant tumors". But there might be also a "disproportionate or *<out-of-step>* development" thanks to "independent progression of characters" (Foulds, 1949b; see also Foulds 1949a). We have seen that transplantable malignant tumors induced by the combined action of carcinogen and goitrogen in the thyroid take in the rat only when thyrotrophic hormone is available in the body. In mice fed with goitrogens the *<out-of-step>* development may acquire aspects so contradictory, from the point of view of the classical definition of malignant growth, as pulmonary metastases of thyroid tissue without there being any histological evidence of neoplasia in the thyroid itself; it is "benign metastasizing" thyroid tissue (Dalton et al. 1949).

One must recall at this place that autonomy, the quintessence of neoplastic cancerous growth according to the classical terminology, has never been clearly defined with reference to growth induced by carcinogens. One must hold in mind that the hydrocarbon which, after having induced a transplantable sarcoma, ceases to act as a stimulus might have called for, or called into existence, a new "stimulus," an other substance** or a virus, harbored in the proliferating cells. One will remember the work of McIntosh who reported in 1933 production of filtrable tumors by the administration of tar to rats and later on also in fowls (McIntosh and Selbie, 1939). Their

* See also the work of Andrews (1950; quoted from Lancet 1950) on "latent prostatic cancer," that is to say prostatic cancer detectable only at microscopical examination of the gland, but already showing definite structural signs of malignancy as abnormal stromal relationship, nuclear changes, mitotic figures, invasion of lymphatics and of blood vessels. A characteristic precancerous hyperplasia affecting particularly glands with benign hypertrophy also is described by this authority.

** The problem of intracellular self-stimuli—supposedly to be evoked by the initiating or promoting agent—has been recently discussed in an encouraging manner by Pullinger (1949). Indeed, no immediate experimental facts are available.

statements were apparently not corroborated (see literature in Foulds, 1937). But subsequent work with birds showed the probability that failure may have been due to production of antibodies able to neutralize the virus, or "masking" the virus (Foulds, 1937; Kidd, 1946; Silber, 1946; see also the summaries of Shabad, 1946, and Kavetski, 1947). Of fundamental interest as to masking is the comparative condition of the spontaneously occurring benign papilloma in the wild cotton-tail rabbit of Shope from which the virus can be extracted, and of the papillomatous or cancerous growth experimentally induced with this virus in the domestic rabbit from which the virus can be recovered only with difficulty or not at all (summary with exhaustive references see Rous, 1943b). "If we did not know the origin of these domestic rabbit lesions, we should record them as tumors of unknown etiology. However, knowing their background we say they are virus-induced tumors" (Duran-Reynals and Shrigley, 1945). There are also the statements of Duran-Reynals and others on the variation of viruses under the influence of the host (Duran-Reynals, 1942; Duran-Reynals and Shrigley, 1945). One cannot avoid admitting the extraordinary relevance which must be attributed, for the whole concept of cancer, to the virus problem which originated as early as 1911 with the famous discovery of Rous of sarcoma in chicken transmitted by virus (summary Rous 1943b). Nothing is changed as to this by the fact that the real place of the virus, including Bittner's milk factor, in the scheme of the evolutional phases of cancer—initiation, promotion and acquisition of autonomy—is unknown to us.

On the other hand, when tentatively discussing the question of the dynamics which might underlay autonomy as acquired by tumoral growth after a certain lapse of time, the findings of Shabad and others with carcinogenic substances to be extracted from cancerous and non-cancerous tissues already referred to also have to be mentioned here.

2. The dynamics of carcinogenic initiation

Upon what kind of action the initiation may rely?

Germinal mutations can be effected with methylcholanthrene in unicellular organisms (Tatum, 1947). In *Drosophila* work has been done with no less than 16 carcinogenic and non-carcinogenic hydrocarbons and azocompounds (Demerec, 1947, 1948); there was seemingly a close correlation between carcinogenic and mutagenic faculties: six out of seven carcinogens tested were mutagenic, while only two out of nine non-carcinogens induced mutations (see also the work of Carr, 1948, Bhattacharya, 1948; Heston, 1949). Indeed, in bacterial mutations which can be effected with various substances there was no such correlation (Latarsek, 1948). There was also the statement that visible damage done by carcinogens to the nucleus is not in proportion to their effect as carcinogens (Darlington and Koller 1947; quoted from Darlington 1948). Of special interest should be here the actions of mustard gas, a most efficient agent of nuclear mutation (work of Auerbach, et al.; bibliogr. in Heston 1949). So far the findings as to its carcinogenic faculties are contradictory. When applied to the skin of mice mustard gas was found not to be an initiating agent (Berenblum and Shubik, 1949a) but it produced pulmonary carcinomas and adenomas and other tumors when injected subcutaneously (Boyland and Horning, 1949) or intravenously (Heston, 1949). The histological effects of nitrogen mustard on the lymphatic nodules of the rabbit are identical in nature with those of X-rays (De Bruyn and Robertson, 1949). The anticarcinogenic faculties of mustard gas are amply known (summary Gilman and Philips, 1946).

On the other hand, Strong (1945, 1947b) made the discovery that malignant gastric

and other lesions are produced in the descendants of mice treated during several generations with methylcholanthrene; these descendants themselves had never received, since up to 6 generations, any carcinogenic chemical. Spontaneous ovarian tumors and a subserous mesothelial reaction as described in our work with guinea pigs also were found in these mice. Strong has interpreted his results as due to a germinal mutation induced by the carcinogenic hydrocarbon. These statements have been substantiated also by some findings of Carr (1948). One may then tentatively assume with different authorities that the local carcinogenic effect of the hydrocarbon—the epithelioma or carcinoma of the skin, or the fibrosarcoma—is due to the induction of a nuclear mutation in the somatic cells on which it is acting. One may explain on these lines the results of Earle (1943) with methylcholanthrene added to tissue cultures which when transplanted into mice develop into fibrosarcoma (Earle and Nettleship, 1943; Nettleship and Earle, 1943; Greene, 1946a).

A new field of an amazing wealth in unexpected facts and fundamental biological implications has been opened with the findings of hereditary transmissible *cytoplasmic* factors of cellular differentiation (summaries of Sonnenborn, 1946; Tatum, 1947). The implication of these discoveries in the cancer problem has been discussed on a broad biological basis by Haddow (1944) and more recently by Darlington (1948) who summarized the results about cytoplasmic particles relative to tumor and cancer development in the following way: (1) there is induction, by chemical agents, of hereditary mutations outside the nucleus and the germ-line; (2) there is a link between hereditary plasmagenes and infectious viruses; (3) these classes of particles are conditional and interchangeable. The origin of cancer may tentatively be ascribed to "mutations in cytoplasmic determinants, indifferently infectious or non-infectious" (Darlington, 1948, p. 125). I have no personal insight into all these intricate problems of cytology, genetics and viruses, and must therefore simply refer to the utterances of the quoted authorities.

3. The evolutional concept, and estrogen-induced experimental growth

How does all this apply to estrogen-induced atypical growth? In discussing this problem which is of fundamental interest for us it seems convenient to start by resuming the essential points we have reached as to the evolution of a neoplasm (table 6).

There is no doubt as to the implication of the genetically determined reactivity of the territory in tumoral growth induced by the continuous action of estrogens. But from here on the difficulties of interpretation begin. Is estrogen acting as a stimulant of initiation or of promotion? The question is of paramount importance. Estrogen-induced abdominal fibroids or endometrial proliferation do not reach the phase of autonomy. Does this mean that estrogen is unable to induce a somatic mutation, and that it is acting exclusively as a stimulant of promotion as in mammary cancer in mice or as androgen in prostatic cancer? It would be idle to speculate about this; there is no possibility of establishing by microscopic examination whether initiation has taken place or not.

There is, on the other hand, the fact that estrogen brings about cancer of the uterine cervix in mice and other cancerous growths (see table 5, p.

105). Has some unknown substance acted here as a stimulant of initiation? This we do not know. But there is still another factor in play when such an estrogen-induced cancer originates: the hormone must have acted for a certain time which seems considerable compared to the life span of the respective species. With X-rays a few seconds may determine the fate of

TABLE 6
The evolution of a neoplasm

1. Genetic determination:

(a) reactivity of the territory:

examples—hereditary mammary carcinoma in mice.

differential response of homologous tissues to estrogens, according to species or strain.

differential response to methylcholanthrene, according to species or strain.

(b) hormonal constitution (?):

example—hereditary mammary carcinoma in mice.

2. Initiation by somatic mutation:

examples—mutagenic action of carcinogens.

milk factor (?) in hereditary mammary carcinoma.

estrogen (?) in different induced cancers.

hypophysial gonadotrophins (?) in testicular and ovarian tumors.*

hypophysial corticotrophins (?) in cortical tumors.*

hypophysial thyrotrophins (?) in tumors of the thyroid.†

3. Promotion, or neoplastic realization:

examples—nonspecific irritants after initial application of hydrocarbons.

estrogen (?) in hereditary mammary carcinoma.

estrogen (?) in different induced cancers.

hormonal imbalance due to the milk factor (?).

hypophysial gonadotrophins (?) in testicular and ovarian tumors.*

hypophysial corticotrophins (?) in cortical tumors.*

hypophysial thyrotrophins (?) in tumors of the thyroid.†

4. Acquisition of autonomous, or irreversible growth.

* See Part III, chapters 18 and 21.

† See p. 112.

the cells (see Part III, p. 232); but in the induction of cutaneous tumors in mice by ultraviolet irradiation the implication of time has been demonstrated (Blum, 1943).

Long duration of estrogenic action is certainly not the only factor responsible of reversible or autonomous tumoral growth. Reference has been made in ch. 3 (p. 38–45) and ch. 6 (p. 82–84) to the tumorigenic action of minute quantities of estrogen if they are allowed to act continuously. In

experiments in which estrogens were absorbed from pellets containing 3 to 10 per cent of estradiol mixed with cholesterol, *epithelial* proliferation of the genital tract was seemingly more pronounced than in former experiments with the injection of large quantities of esterified hormones.

One may tentatively assume that long and continuous action of *very small* quantities of estrogen are those experimental conditions which are most propitious to bring about the change in the behavior of the glandular

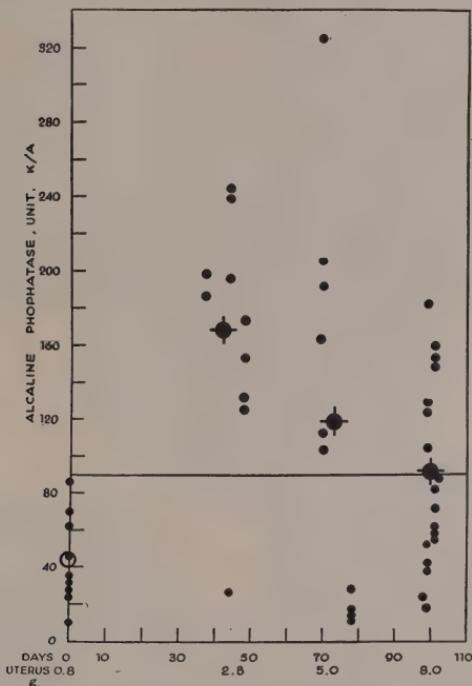


FIG. 63. Increase of alkaline phosphatase in the uterus of guinea pig. Benzoestrol diprop. 37 to 101 days. Abscissa—duration in days and average uterine weight. Ordinates—King-Armstrong units. At 0 uteri of normal animals. Horizontal line at 90—normal limit. Phosphatase was determined in the thickened uterine mucosa and submucosa separated from the myometrium. Greatest increase at 37 to 48 days. At about 70 days there is again a considerable number of animals with normal values, especially at 100 days. Large black circles with cross—average of the respective group.

cells of the endometrium which leads to invasion. This would explain why cancerous growth may be observed in aged women when ovarian endocrine activity is in its decline. So far this is only a tentative suggestion. But various experimental findings render probable that under certain quantitative conditions, i.e. when large quantities of estrogen are administered, continuous action is likely to diminish the proliferative faculty of the uterine and other epithelia. This may be exemplified by the occurrence of phosphatases in the uterine mucosa subject to the prolonged action of

estrogen (studied in our Department since 1943 by Fuenzalida and collaborators; Fuenzalida, 1949). A considerable increase of alkaline phosphatase takes place in the uterus in the first 6 to 8 weeks (Sarras, 1944); though both the myometrium and the mucosa thicken the increase is limited to the proliferated mucosa (Pinto, 1946). But when the experiment was prolonged for several months the alkaline phosphatase in the epithelium began to diminish again though without reaching the normal level (fig. 63). One may then assume that, as time goes on, the uterine epithelium is damaged by the estrogen and rendered less capable of proliferative processes.

The toxic action of estrogen on the uterine epithelium and other parts of the uterine wall has been evidenced also in work related to pyometra which has been observed in mice, rats and rabbits estrogen having been admin-

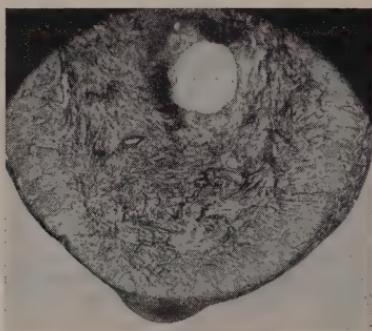


FIG. 64. Complete necrosis of endometrium and of part of glands. No pyometra and no leucocytic infiltration. 38 inj. of 500 µg of stilbestrol, 104 days. Endometrium absent. The dark patches in the submucosa are "fibroids" without sharp limits. To the right—thickened subserosa with smooth muscle fibers; comp. to fig. 16E. On the ventral side a small subserous fibroid. $\times 5$. (II.St.8).

istered for a sufficient length of time (references Weinstein et al., 1937; Allen et al., 1939, p. 576; Zondek, 1936). We have made a detailed study of the related phenomena in the guinea pig. The uterine epithelium may disappear (figs. 64 and 65). There may be also necrosis of the uterine submucosa (figs. 65 and 66) and even of the myometrium. Indeed, I must lay stress on the fact that necrotizing actions of the estrogen on the different tissues of the uterine wall were produced in our work also with small quantities of the estrogen when allowed to act continuously; but so far we have not studied the question whether the incidence of necrosis depends on the quantities administered.

Mammary adenocarcinoma in mice may fail to appear when quantities of estrogen sufficiently large are given (Allen et al., 1939, p. 569-570); incidence in male mice of cancerous strains can be brought down from 70

per cent to as little as 18 per cent by increasing the quantity of estrogen administered (Gardner, 1941).

The above statements will make it clear that experimental reproduction of tumoral structures known from human pathology cannot be an easy

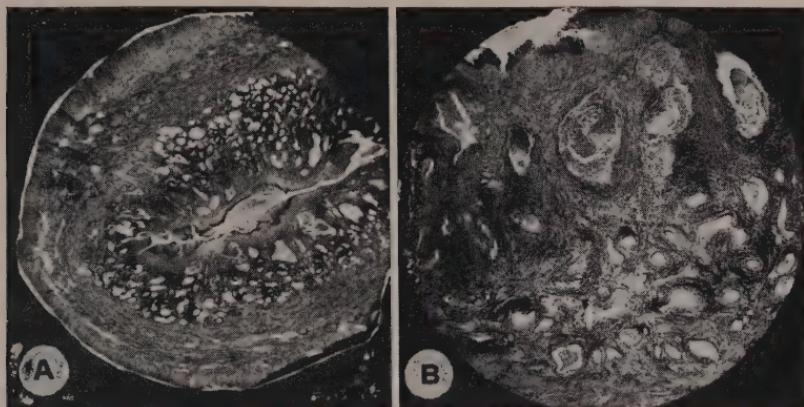


FIG. 65. Complete necrosis of endometrium and of part of the submucosa. Pyometra. Epithelium of uterine glands in the upper part of the submucosa has disappeared and the glands are filled with pus. 99 inj. of 20 μ g of estrad. (benz.), 215 days. A. \times 5. B. \times 23. (I.7).

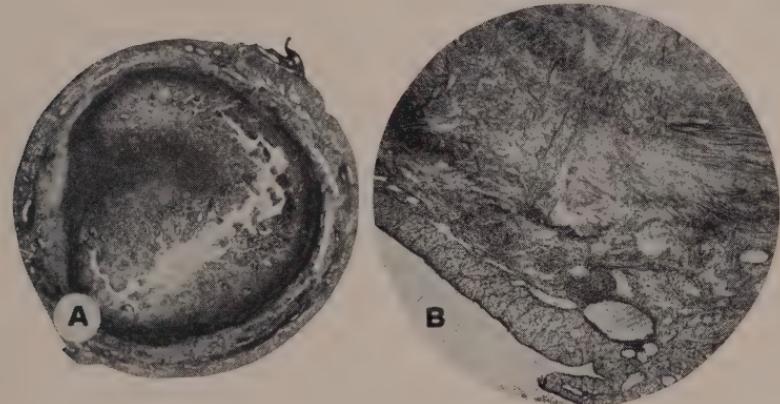


FIG. 66. Complete necrosis of endometrium and of submucosa, and of part of the myometrium. Pyometra. Thick leucocytic infiltration of submucosa and of myometrium. Longitudinal layers of myometrium intact. 53 inj. of 40 μ g of estrad. (benz.), 122 days. A. \times 5. B. \times 23. (I.9).

task. In work with estrogen-induced fibroids, for instance, the tumor is induced under quantitative and timing conditions which are probably very different from those prevailing in the body of the woman in which a fibroid originates spontaneously. An overthrow of the steroid balance also might

be in play (Part III). This may explain why the structure of the experimental fibromyoma is often so different from that of the spontaneous one.²

The fact that estrogen-induced tumoral growth is mostly reversible is often taken as a proof that estrogen by itself is unable to induce cancer, and that such growth differs fundamentally from spontaneous cancer. This idea, besides overlooking the fact that cancer, though rarely, can be induced experimentally by estrogen, is intimately linked with the old concept that neoplastic growth, being—as it really is—fundamentally different from localized hyperplasia, is autonomous and irreversible *from its very beginning*. The concept was based on the anatomical, histological and clinical observation of a limited number of types among neoplasms in man. However, what do we know about the causative stimuli of neoplasms in man when due allowance is made for occupational tumors? How can the statement be made that further growth of a spontaneous neoplasm is independent of the causative stimulus when the latter is completely unknown to us? On the other hand, there is now clear cut knowledge on the gradual evolution of autonomy in animal and human neoplasms, including cancer. There is ample knowledge on the reversible and irreversible proliferative processes being intimately linked one with another in experimental epithelioma induced by hydrocarbons. There is the possibility that the process of promotion is operated by a different agent than that of initiation in spontaneous cancer as is most probably the case in prostatic carcinoma in man, or in mammary carcinoma in mice. There is much experimental evidence that the length of action of a promoting agent is important for establishing autonomous tumoral growth, and there is likewise certain clinical evidence that endometrial glandular hyperplasia due to the prolonged action of endogenous estrogen may lead to adenocarcinoma in women. We do not even know whether the estrogen which has acted as the stimulus of hyperplasia really ceases when the malignant degeneration starts. According to new statements the histological condition of the ovary is in endometrial carcinoma significantly different from that in normal women especially as to the behavior of the interstitial tissue (Woll et al., 1948). This tissue has been seemingly underrated as to its hormonal significance in women (for the rabbit see Lipschutz, 1924; with references also to the important older work of Bouin, Limon et al.). And the ovary is not the

² Hamblen (1945, p. 529) referred to our statement that quantities of estradiol 100 to 150 times greater than the hysterotrophic or physiological dose are necessary to induce fibroids in the guinea pig. But this is true only for experiments in which the hormone is given by injection. Things change completely when the hormone is absorbed continuously from a subcutaneously implanted pellet. Then the quantity necessary to produce fibroids is many times smaller than the above figure, though certainly greater than the hysterotrophic dose (Thibaut, 1941; Lipschutz, Thibaut and Vargas, 1942; see chapter 3).

only source of estrogen in the body! There is the probability that cystic hyperplasia of the mammary gland—or chronic cystic mastitis—is a frequent precursor of mammary cancer in women (work of Geschickter; Cole and Rossiter; quoted from Prudente, 1945; Willis, 1948, with ample literature; contrary to this concept are the statements of Pullinger, 1947). There is clear cut knowledge on the fact that the further growth of uterine fibromyoma in women is bound to the action of estrogen in whose absence this tumor ceases growing or even shrinks. There is clear cut knowledge on the fact that prostatic cancer in man is bound at the beginning, though already malignant, to the action of androgen, both tumor and metastases being reversible; and this phase of reversibility is followed *only afterwards* by a phase of irreversibility.

The old rigid concept of the neoplasm being at its very start autonomous and irreversible and fundamentally different from reversible growth, is seemingly at variance with all this new knowledge, experimental and clinical, to which due allowance has to be made in a modern concept of neoplastic growth.

PART II.

The Antitumorigenic Action of Steroids

Chapter 10.

ANTAGONISTIC ACTIONS OF SEX HORMONES

The antitumorigenic action of steroids is intimately linked with an old problem of sex endocrinology: the antagonism of sex hormones; a problem which has been raised forty years ago by classical findings of Steinach, and subsequently also of Sand, with the transplantation of the sex glands into animals of the opposite sex, or with the simultaneous transplantation of the glands of both sexes into the same animal (summaries Steinach and Kun, 1926; Sand, 1933). In our own work in this field the conclusion was finally reached that there is an antagonistic play of the hormones of the two sexes in the receptive territories, that is to say, that the sensitivity of the receptive tissue and the response toward the hormone of the proper sex may be diminished by the simultaneous action of the hormone of the opposite sex. This conclusion was supported not by injecting sex hormones which were not yet available in those times, but by grafting ovaries into males, under variable experimental conditions. In similar experiments in which both ovarian and testicular tissues are simultaneously present in the body, characters of both sexes (mammary glands and penis) may be fully developed. But cases also occurred in which the male characters were of the castrate type though testicular tissue with interstitial gland and seminiferous tubules of normal aspect was found; or in which there was no development of the mammary glands though the ovaries contained large Graafian follicles as active ovarian grafts generally do.¹ From these statements we drew the conclusion that under certain quantitative conditions the ovarian hormone may inhibit the action of the testicular hormone, and *vice versa*.² Though full corroboration was given to our clear cut experimental statements (Smelser, 1933), our conclusion met in bygone times with almost unanimous disapproval.³ Several authorities quite mistakenly

¹ The last mentioned statement has been made already by Sand (1919).

² The results of this older phase of our work have been described in Endocrinology, **9**: 109. 1925; J. of Physiol., **58**: 461. 1924; **59**: 333. 1925; C. R. Soc. Biol., **89** to **97**. 1923 to 1927; Pflügers Arch. für die ges. Physiol., **207**, **208**, **211** and **221** (1925 to 1926 and 1929). See also Lipschutz (1927) and chapter IV of Lipschutz, "La Autorregulación Orgánica," Edit. Morata, Madrid, 1930 (the chapt. "El problema del antagonismo de las glándulas sexuales y su relación con la patología").

³ The persistent refusal, especially between the two World Wars, of the basic

confused our conclusions about the peripheral antagonism of sex hormones with a fundamentally different phenomenon: the resistance the gonad of the host offers against the taking and functioning of the ovarian graft, a phenomenon discovered by Steinach and subsequently shown to be related to the anterior lobe of the hypophysis. The resistance can be overcome when, for instance, male rats with ovarian grafts are given hypophysial gonadotrophic hormones (Engle, 1929; Moore and Price, 1932). These gonadal-hypophysial relationships have nothing to do with the peripheral antagonistic play of the gonadal hormones. But there were also other objections against our concept. As we found a guinea pig with an intact testicle and an ovarian graft may have mammary glands with milk secretion and at the same time may be able to impregnate successfully the female. This coexistence of fully developed male and female characters as observed sometimes in similar experimental work, was thought by many authorities to be by itself sufficient to show that there was no antagonistic play of sex hormones. Later on, when chemically pure hormones became available coexistence of male and female sex characters was obtained also by the simultaneous administration of androgens and estrogens. But the authorities overlooked the fact that all these statements were quite insufficient to invalidate our concept of a peripheral antagonism of the sex specific hormones, since the result must necessarily depend on the quantities of the hormones in play, as we had to assume from the beginning and as has been corroborated by all subsequent work. The epidermization of the vaginal mucosa induced in the castrated rat by estrogens has been promptly antagonized by the simultaneous administration of testosterone (Courrier and Cohen-Solal, 1937a); the estrogen-induced metaplasia in the reproductive accessory organs of male rats and mice has been inhibited by the simultaneous administration of androgens (Korenchevsky and Dennison, 1935; Rusch, 1937; Harsh et al., 1939), and the same was found in dogs (De Jongh et al., 1939) and in anthropoids (Zuckerman and Parkes, 1936). Vice versa, the growth of the comb in the capon as induced by androgen has been antagonized by the administration of estrogen (Gley and Delor, 1937; Emmens, 1939; Mühlbock, 1938-1940), even when applied directly to the comb (Hoskins and Koch, 1938; Morató-Manaro and Albrieux, 1938). The concept of an antagonistic play of sex hormones in the receptive tissues has been applied also to the mutual relations between the steroid

concepts of modern sex endocrinology, including the antagonism of sex hormones, was due not to intrinsic scientific moments but to a socio-psychological complex being meddled with, and this complex was very far indeed from being a page of honor in the history of modern science. Some of these aspects have been analyzed in a lecture delivered in Strasbourg as a homage to Professor P. Bouin. See Arch. d'Anat., d'Histol. et d'Embr. 30: 119. 1947.

hormones of the same sex as estrogen and progesterone. The estrogenic action on the vaginal mucosa has been antagonized in the castrated rat by luteal hormone (Courrier and Cohen-Solal, 1937b; see also Allen and Meyer, 1935); likewise the progestational action of progesterone in the rabbit has been antagonized by estrogen (Robson, 1936).

There is to-day no doubt any more about such an antagonistic play of the sex hormones. This concept has been applied by various authorities also in work with estrogen-induced and spontaneous neoplastic growth. A systematic investigation of these aspects of sex hormone antagonism has revealed its fundamental importance both for physiology and pathology. Our work with the antifibromatogenic action of steroid hormones we shall deal with in this Part has been but an application of the concept of the antagonistic play of sex hormones.

Chapter 11.

COMPARATIVE ANTIFIBROMATOGENIC ACTION OF DIFFERENT STEROIDS

A. ANTIFIBROMATOGENIC ACTION OF PROGESTERONE

The statement that abdominal fibroids were so easily elicited in female guinea pigs to which estrogen was administered for a sufficient length of time posed the question of why fibroids failed to appear in our former work with ovarian fragmentation (Lipschutz, 1936a, 1937, 1938). In these animals prolonged follicular phases appeared, with persisting cornification of the vaginal mucosa, mammary development, sometimes monstrous growth of the uterus, atypical proliferation of the cervical mucosa and the endometrium, and with uterine bleeding (see p. 184). But there were no fibrous nodules, though, as we know to-day, they may be elicited also with endogenous estrogen (see p. 33). It is true, uterine and mesenteric fibromyomas have been found in two animals with ovarian fragmentation (Morató, 1941; Nadel, 1949). But these cases were exceptional. I assumed that the intercalation of luteal phases as evidenced by the microscopical condition of ovarian fragments, was responsible of there being no fibroids in most of the animals with ovarian fragmentation. Several workers have tried to prevent with progesterone and testosterone the proliferative action of estrogens on the genital tract (see references, table 15; p. 161). In our work the estrogen-induced abdominal fibroid was used as a convenient test for the antitumorigenic action of progesterone and other steroids.

Abdominal fibroids failed to appear when progesterone was injected simultaneously with estrogen (Lipschutz, Murillo and Vargas, 1939; Murillo, 1940; Lipschutz and Vargas, 1941b). In experiments which lasted 3 months, the preventive action was in general overwhelming, only small nodules being still present on the surface of the spleen and the surrounding parts of the stomach and abdominal wall, and so also some fibrous strands. One will remember that these fibrous reactions are first to appear when small doses of estrogen are given; they are the last to disappear when progesterone is given simultaneously with estrogen.

B. THE ANTIFIBROMATOGENIC DIAGRAM

One of the first questions we had to deal with was that of the *quantity* of progesterone necessary for preventing estrogen-induced fibroids. We

had to start from the existing knowledge on the quantities of progesterone able to antagonize the estrogen-induced epidermization of the vaginal mucosa—the two-hundredfold of progesterone according to Courrier and Cohen-Solal (1937b). Consequently we injected in our first experiments quantities of progesterone 150 times those of the simultaneously injected benzoate of α -estradiol: a complete or almost complete prevention of experimental fibroids took place. Uterine fibroids were always completely absent. But the quantitative aspect underwent a sudden change when, instead of injections, Deanesly and Parkes' technic of subcutaneous implantation of pellets was used in our work. The total absorption of the steroid varies according to the surface of the pellet; about 18 to 48 mg of progesterone were absorbed in the course of 50 days. With these quantities of progesterone the fibromatogenic action of 1 to 5 mg of α -estradiol also absorbed from subcutaneous pellets was prevented (Lipschutz and Vargas, 1941b; González, 1943). This result seemed to be extraordinary when compared with the estrogen-progesterone ratio in injection experiments, and was a potent stimulus to study in greater detail the quantitative relations of the fibromatogenic and antifibromatogenic steroids.

Expressing the afg.¹ faculty of progesterone or other steroids as a multiple of the fibromatogenic estrogen absorbed simultaneously was soon revealed to be an oversimplification. According to our old concept action of a sex hormone is antagonized by an other sex hormone because the latter desensibilizes the receptive cell or tissue on which the first one normally acts. The question arose about the quantitative conditions upon which the supposed desensibilization may depend. It was the question whether the effect was dependent on a *threshold quantity* of the antagonizing hormone. The question was examined in the following experiments (Lipschutz, Luco and Zañartu, 1942). The fibromatogenic average of 2.3 mg of α -estradiol absorbed from subcutaneously implanted pellets in the course of 2 months was antagonized with 6.8 mg of desoxycorticosterone, that is to say, at a ratio of 1:3 (see table 7). When the quantity of estradiol was raised from 2.3 to 7.8 mg, antagonization was still effected with 10 mg of desoxycorticosterone, that is to say at a ratio of only 1:1.3. But on the other hand, with this ratio the same clear cut effect was no more obtained in experiments in which the quantity of estradiol was of 2 mg, as in the first group, but the quantity of desoxycorticosterone was reduced to only 3.1 mg. In other words: a certain minimum quantity of the afg. steroid seems to be necessary for the prevention of the fibromatogenic action of estrogen. This threshold quantity may be expressed in micrograms absorbed per day. In the above experimental series the threshold quantity of desoxycorticosterone acetate was of about 90 μ g per day (calculated as the free steroid) though with smaller quantities some afg. action may be still obtained.

¹ Abbreviation of antifibromatogenic.

Similar quantitative conditions prevail seemingly also in other instances of sex hormone antagonism as the inhibition of the estrogen-induced growth of the oviduct in chicks by progesterone (Hertz et al., 1947; see details below).²

Special stress must be laid on the fact that all these figures, and those to which reference has to be made in the following, are *but very approximate*. Instead of 80 to 90 µg prevention was obtained in an other series already with half of this amount. These considerable discrepancies are due to manifold technical factors. Calculation of daily absorption by dividing the total absorption by the number of days is but a very rough procedure; even the total quantity absorbed in the course of the experiment and calculated from the loss of weight of the pellet, is only approximate (Deanesly and Parkes, 1943; Folley, 1943; Deanesly et al., 1946; Cowie and Folley, 1946). Of even greater importance is the fact that the quantitative classification of the estrogen-induced tumoral reaction as described in the Part I (p. 38) re-

TABLE 7

Group*	Estradiol	Desoxycorticosterone acet. [†]	Ratio of steroids	Antagonization
I	mg 2.3	mg 6.8	1:3	+
II	mg 7.8	mg 10.0	1:1.3	+
III	mg 2.0	mg 3.1	1:1.6	±

* Experiments lasted 2 months.

† Figures calculated for the *free* steroid.

vealed to be but a very rough method in experiments with the simultaneous administration of other steroids. As already insisted upon, tiny nodules on the surface of the spleen—the tumoral seed—and some fibrous strands of a variable abdominal localization were still present in experiments lasting 3 months, notwithstanding the otherwise preventive action of progesterone. We were unable to reach a definite concept about what would be best to consider as the “end point” of afg. action, and I never could overcome a feeling of uneasiness when giving a figure for the afg. threshold. All the more, as soon the conviction was reached that with quantities smaller than the supposed threshold some preventive action, though a very incomplete one, also may be obtained (fig. 69A). And nevertheless applying the notion

² Whether these quantitative conditions have general application cannot be said with certainty; the reader may be remitted to the work done with the inhibition of the progestational action of progesterone on the uterine mucosa by estrogen in the rabbit (literature in Gilman and Stein, 1942; Courrier and Poumeau-Delille, 1943-1945; Jost, 1944), and in anthropoids (Hisaw, 1935; Courrier and Gros, 1937; Gilman and Stein, 1941).

of an afg. threshold quantity with figures of a very doubtful precision was extremely helpful in achieving progress in further comparative work with different steroids.

For a diagrammatic expression of our results we have used what may be called the "antifibromatogenic diagram" (fig. 67). On the abscissa quan-

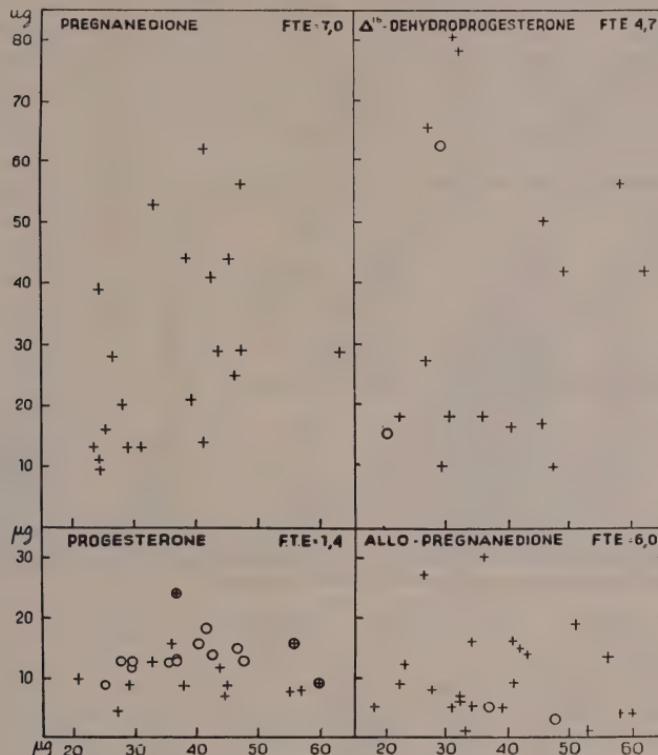


FIG. 67. Antifibromatogenic diagrams of four different steroids. Quantities of progesterone and other steroids absorbed daily from subcut. pellets are plotted as ordinates, against estrad. as the abscissa. +, animals with fibroids; O, animals without fibroids; \oplus , animals with some fibroids of about 1 to 1.5 mm in diameter, or with notable fibrous strands. With 10 to 20 μg of progesterone daily most animals were without fibroids. The remaining three steroids were not afg. with the quantities used.

tities of estrogen absorbed per day are given; the quantities of the afg. steroid are given as ordinates. All animals in which the average absorption of progesterone was less, or no more, than 10 μg per day showed abdominal fibroids. This result is independent of the quantity of estrogen per day; as already explained (p. 38) the quantities of estrogen sufficient to elicit fibroids are much smaller than those in the diagram. With about 20 μg of progesterone per day fibroids were prevented in most of our animals; only

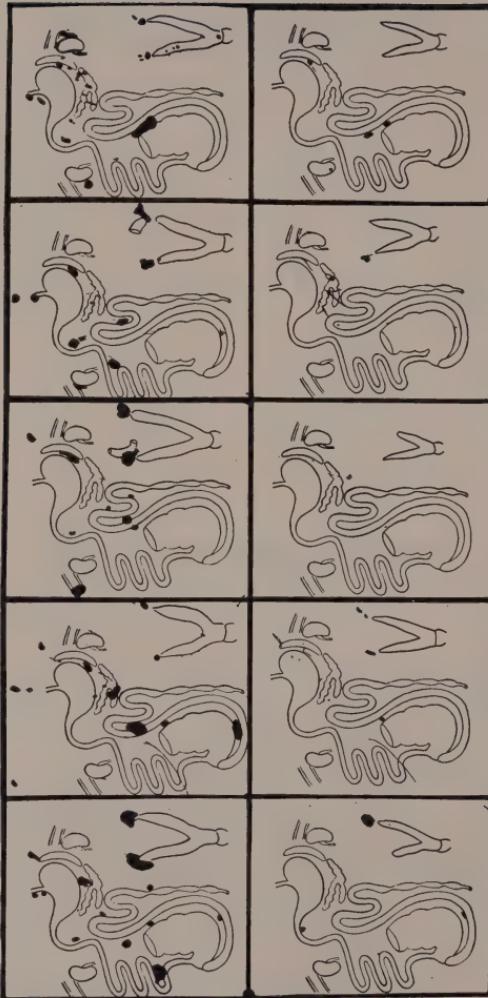


FIG. 68. Comparative fibromatogenic reaction in animals with estradiol alone, and animals with estradiol and an antifibromatogenic steroid (dihydrotestosterone). Duration—90 days. Diagrams taken at random from the protocols. Row above—13, 28, 31, 32 and 33 μg of estrad. alone per day. Uterine weights: 5 to 6.5 g (CVII.12.13.19.3.5). Row beneath—33 to 40 μg of estrad. and 76 to 100 μg of dihydrotestosterone per day. Uterine weights: 2.1 to 3.0 g (C-VII.dt.14.9.11.10.12). The quantities of the afg. steroid were yet unsufficient to antagonize completely the fibromatogenic action of the estrogen but there was already a pronounced afg. action. The diagrams demonstrate also the difficulty one is meeting with when trying to give exact figures for the afg. threshold quantity of a steroid.

small un conspicuous nodules were still present. Often we did not feel sure about whether a small nodule was to be classed as 0.5 or 1, in such a way that the appreciation is falsified in many cases. The figures of 15 or 20 µg might well be 30 or 40! But notwithstanding all these drawbacks the results are clear cut. There is the remarkable fact that the quantity of progesterone which was able to antagonize the fibromatogenic action of estrogen was often even less than the quantity of the latter (González, 1943; Lipschutz, Bruzzone and Fuenzalida, 1944). There can be no more spectacular demonstration of the fallacy, from a quantitative point of view, of our first experiments in which estrogen and progesterone were injected at a ratio of 1:150!

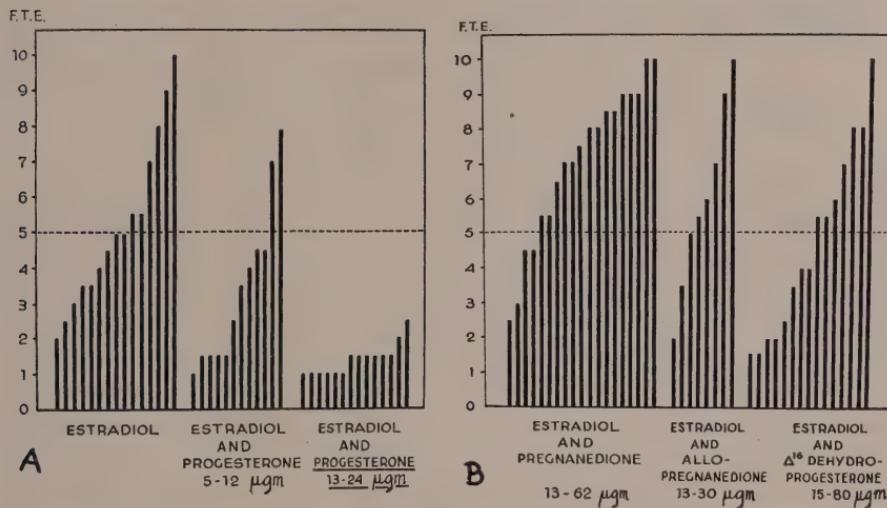


FIG. 69. A. Afg. action of small quantities of progesterone, subcut. pellet (40 per cent of progesterone). Absorption calculated on the assumption of non-selective absorption; total absorption divided by number of days. Duration—3 months. Horizontal line—average F.T.E. of the estradiol group. With 13 to 24 µg of progesterone the afg. action is very considerable; smaller quantities may have some protective action. B. Failure to induce an afg. action with 3 other 3-keto-steroids. No diminution of the F.T.E.

An example taken at random (fig. 68) gives an idea of our comparative work with afg. steroids, and shows at the same time the drawbacks we encountered when prevention was not so far going as in experiments with progesterone.

We tried to express results also in other ways. One may give in a diagram the fibrous reaction of each animal in units of our system of classification (see fig. 69A). The difference between the estradiol group and the estradiol-progesterone group is striking and convincing (Lipschutz, Bruzzone and Fuenzalida, 1944). Three facts of fundamental interest have to be insisted upon when comparing fig. 69A and fig. 67. *First*, as already men-

tioned on preceding pages and as again shown in fig. 69A, prevention was not complete with what has been called the afg. threshold quantity, small nodules still being present on the surface of the spleen; it may be added that prevention was often incomplete even with quantities of progesterone much greater than that (Lipschutz and Vargas, 1941b). *Second*, quantities of progesterone smaller than the threshold also may exert some preventive action (fig. 69A). *Third*, the preventive action of a given quantity of pro-

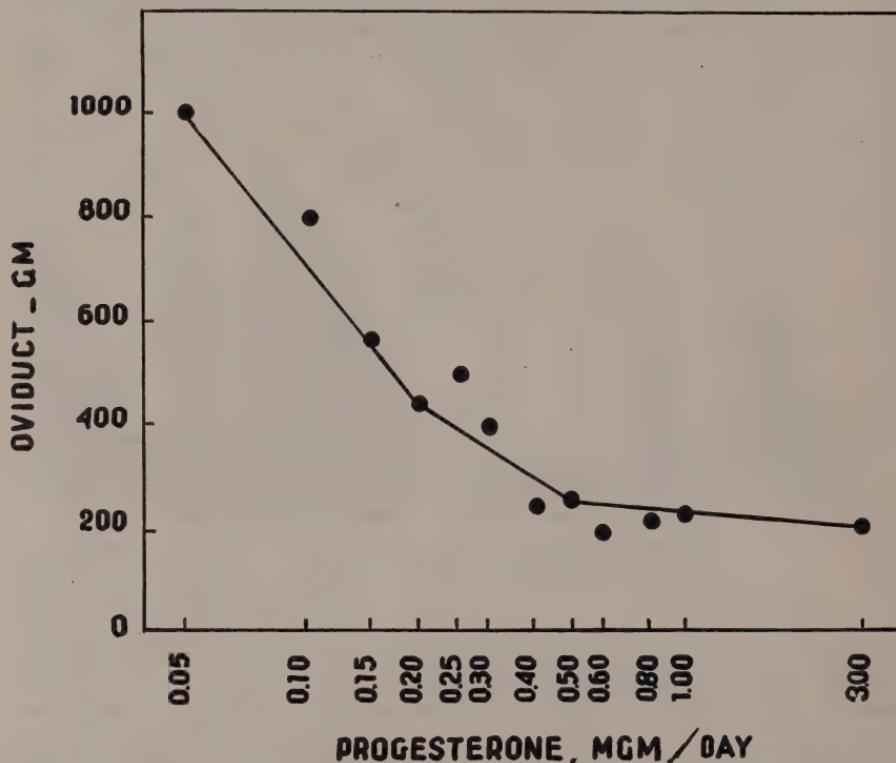


FIG. 70. Inhibition of estrogen-induced growth of the oviduct of chicken by different doses of progesterone. The degree of inhibition increases with greater doses of progesterone; but with doses between 0.4 and 3.0 mg no increase of action is obtained. From Hertz *et al.*

gesterone was independent of that of the simultaneously administered estrogen (fig. 67). These "quantitative" findings of ours which could not be but of so doubtful a precision, are substantiated by remarkable recent statements of Hertz *et al.* (1947) about the quantitative laws underlaying the inhibition of estrogen-induced growth of the oviduct in chicks with progesterone. There was a steep increase of inhibition from 0 to 75 per cent with 50 to 600 μ g per day; inhibition increased only slightly, or did not

increase at all, when the quantity of progesterone was raised from 600 to 3000 μg per day. Even with these large quantities of progesterone the weight of the oviduct remained still greater than without estrogen: there was with the oviduct a "basal amount" of estrogen-induced growth resistant to progesterone inhibition, and an "additional growth response" quantitatively sensitive to increasing doses of progesterone (Hertz et al., 1947). And finally: it made scarcely any difference when the estrogen dosage was increased as much as the 16-fold the inhibitory effect with progesterone remaining the same. The logarithmic curve by which the quantitative results of Hertz et al. with the oviduct are expressed (fig. 70) could be made use of tentatively as a diagram illustrative of the quantitative interpretation we have given to our results with the prevention of estrogen-induced fibroids as exemplified in fig. 69.

C. ANTIFIBROMATOGENIC ACTION OF TESTOSTERONE AND DESOXYCORTICOSTERONE

In our former work on the antagonism of sex hormones before the latter were chemically isolated, androgen was considered as the classical antagonist of the follicular hormone (p. 125). Now, testosterone and its propionic ester were shown to be also afg. But from the beginning it became evident that their activity was considerably smaller than that of progesterone. Whereas fibroids were prevented with about 20 μg of progesterone more than 100 μg of testosterone, absorbed from a subcutaneously implanted pellet of the propionate, were necessary. There were sometimes conflicting results. There was in work with pellets containing 40 per cent of testosterone and 60 per cent of cholesterol apparently prevention when only 40 to 80 μg per day were absorbed whereas fibroids still occurred even with as much as 200 or 250 μg per day (Iglesias and Lipschutz, 1944). Under these circumstances it was not possible to give a reliable figure for the afg. threshold of testosterone. But the results give sufficient evidence that, *first*, with testosterone, free or esterified, greater quantities are necessary than with progesterone; and *secondly*, that prevention is less constant and less uniform with testosterone compared to progesterone.

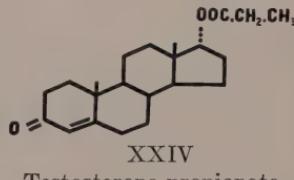
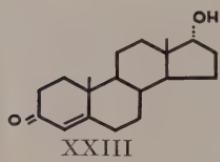
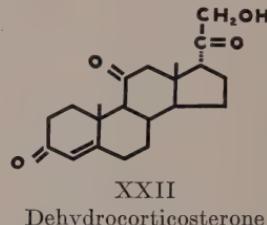
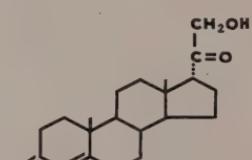
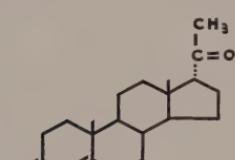
Reference has already been made to the afg. action of a cortical hormone as desoxycorticosterone (Lipschutz, Vargas and Nuñez, 1941; Nuñez, 1942). As already mentioned the threshold quantity was with this steroid of about 90 μg per day and in some series less than that; the acetate was used in most of the experiments.

The difference between the three mentioned steroids as to their comparative afg. activity was considerable and striking, and far beyond the possibilities of error.

Chapter 12.

STRUCTURAL PARTICULARITIES OF ANTIFIBROMATOGENIC STEROIDS

Considerable progress was obtained in our work when we tried to correlate afg. activity of steroid compounds with chemical structure.¹ We have hitherto referred to afg. activity of three steroid hormones; a fourth steroid hormone occurring in the body, dehydrocorticosterone, also was found active (fig. 71) (Lipschutz and Zañartu, 1942; Zañartu, 1942). All these afg. steroids were 3-keto-compounds with a double bond in ring A. There



was also the statement that progesterone and the two cortical hormones were more active than testosterone. The question arose whether afg. activity was enhanced by the side chain of two carbons at C₁₇. We have made a systematic search among known steroids, occurring or not occurring in the body, with the special purpose of studying the question how far afg.

¹ For the question of interrelations existing between chemical structure of steroids and their physiological and pharmacological actions in general the reader may be referred to the exhaustive chapter of Selye (1947b, p. 69-73) and to former papers of the same author and associates (Selye, 1942a, 1942b; Albert and Selye, 1942; Selye and Masson, 1943; Haour, 1948; Haour and Selye, 1948).

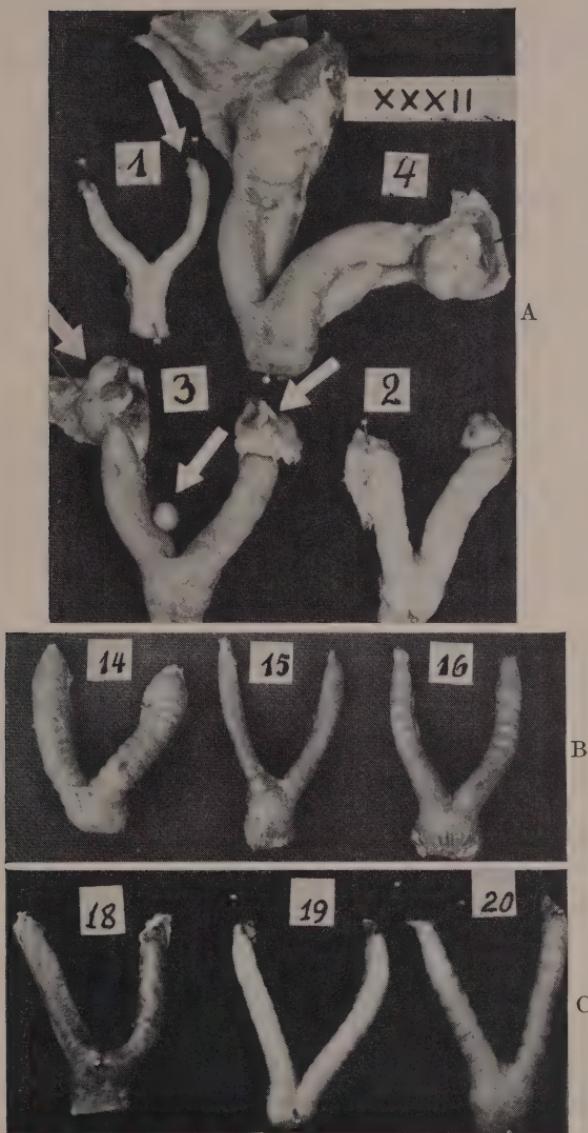


FIG. 71. Afg. action of cortical steroids. A. 1 to 4: Uteri of four animals with estrad. alone. 11 to 57 μ g of estrad. per day, subcut. pellet, 70 to 73 days. In 1 the pellet was not found at necropsy. Note parametrial and apical tumors. B. 14 to 16: Uteri of animals with estrad. and desoxycorticosterone acet., 18 to 44 μ g of estradiol and of 59 to 100 μ g of desoxycorticost. per day, 70 to 73 days. Uteri much smaller and no tumors. C. 18 to 20: Uteri of animals with estrad. and dehydrocorticost. 17 to 57 μ g of estradiol and of 68 to 100 μ g of dehydrocorticost. daily, 69 to 71 days. Uteri much smaller and no tumors. (XXXII).

activity may be influenced by chemical structure (Lipschutz, 1944, 1946, 1947). Twenty three different steroids and five esters of these were examined. Results obtained in 1941 to 1948 are summarized in table 8.

The definite statement can be made that afg. activity of steroids is intimately related to the ketonic group at C₃. Various compounds with an hydroxyl group at C₃ were examined: androsterone (Watt, 1943); Δ⁵-androstenediol and androstanediol (Iglesias, unpubl.); 21-acetoxypregneno-lone (Lipschutz, Bruzzone and Fuenzalida, 1943); pregnenolone acetate

TABLE 8

	Antifibromatogenic (3-keto-steroids)	Not antifibromatogenic (3-keto-steroids)	Not antifibromatogenic (OH at C ₃)
Occurring in the body (or their esters)	With Δ ⁴ : (1) progesterone (15-20) (2) desoxycorticosterone (40- 90) (2a) desoxycorticosterone acetate (40-90) (3) dehydrocorticosterone (<100) (4) testosterone (>150)* (4a) testosterone propionate (>130)*		(17) androsterone [110] (17a) androsterone benzoate [240] (18) Kendall's comp. H(17- ethyl-androstene-3,21- diol-11,20-dione) [150?] [†]
Not occurring in the body	(5) 17-methyl-testosterone (150) (6) 17-ethynil-testosterone <td>With Δ⁴: (9) Δ¹⁶-dehydroprogesterone [160] (10) 17-ethyl-testosterone [>210?]* (11) 17-vinyl-testosterone [>350?]* (12) androstene-3,17-dione [570] (13) cholestenone [160] Without Δ⁴: (7) dihydrotestosterone (>110) (7a) dihydrotestosterone propionate (>150) (8) 17-methyl-dihydrotes- tosterone (>110)</br></td> <td>(19) androstan-3,17-diol [46] (19a) androstan-3,17-diol [220] (20) Δ⁵-androstene-3,17-diol [175] (21) 17-ethyl-Δ⁵-androstene- 3,17-diol [30] (22) Δ⁵-21-acetoxypregnene- 3-ol-20-one [1240][†] (23) Δ⁵-pregnene-3,21-ol-20- one-3-acetate [500][‡]</td>	With Δ ⁴ : (9) Δ ¹⁶ -dehydroprogesterone [160] (10) 17-ethyl-testosterone [>210?]* (11) 17-vinyl-testosterone 	(19) androstan-3,17-diol [46] (19a) androstan-3,17-diol [220] (20) Δ ⁵ -androstene-3,17-diol [175] (21) 17-ethyl-Δ ⁵ -androstene- 3,17-diol [30] (22) Δ ⁵ -21-acetoxypregnene- 3-ol-20-one [1240] [†] (23) Δ ⁵ -pregnene-3,21-ol-20- one-3-acetate [500] [‡]
		(14) allo-pregnane-3,20- dione [150] (15) pregnane-3,20-dione [260] (16) methyl-dehydrocholate [1060]	

() Prevention with this quantity in μg; [] no prevention with this quantity, in μg.

* Inexact figures: see text.

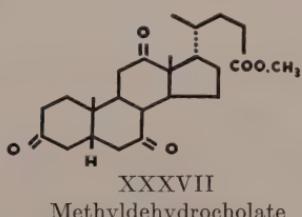
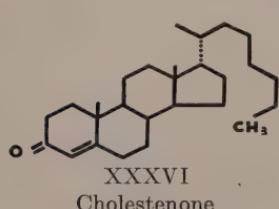
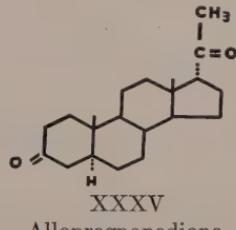
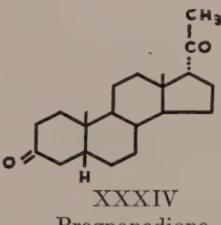
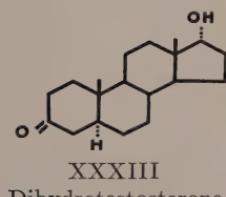
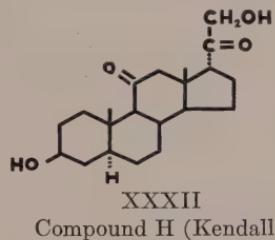
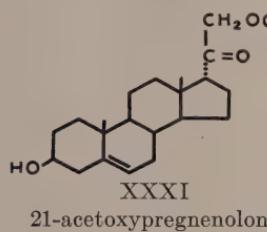
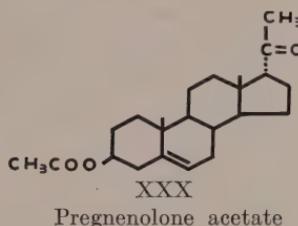
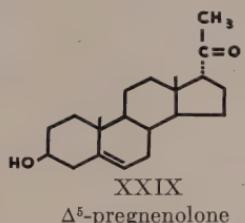
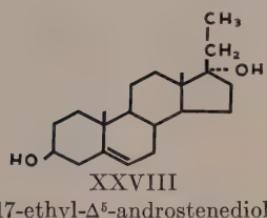
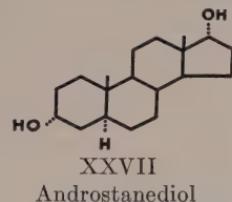
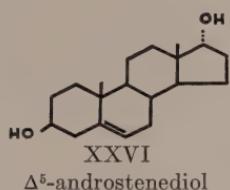
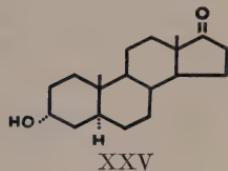
† Slight antifibromatogenic action with these large quantities.

‡ Antifibromatogenic action with 550 to 1670 μg.

(Iglesias and Bruzzone, 1948); Kendall's compound H (Lipschutz and Zañartu, 1942); and 17-ethyl-Δ⁵-androstenediol. None of these was able to prevent fibroids under quantitative conditions comparable to the four mentioned 3-keto-steroids.

The two pregnenolone derivatives offer special interest. Since progesterone and desoxycorticosterone are more active than testosterone, the question arose whether a compound with an hydroxyl at C₃ may acquire

afg. activity when a side chain of two carbons as in the two hormones was added at C₁₇. With an average of 800 to 1240 µg of 21-acetoxypregnenolone per day a slight diminution of the fibromatogenic effect of estradiol was



obtained; pregnenolone acetate became afg. with quantities as large as 550 to 1670 μg per day (Iglesias and Bruzzone 1948). None of these results is able to shake the statement about the ketonic group at C₃ being fundamental for the afg. activity of steroid compounds. The unexpected finding with the afg. activity of the two pregnenolone derivatives when quantities about 50 to 100 times those of progesterone are given, may be compared

List of Steroids used in our work on the antifibromatogenic action

	M.P. Bibliography	M.P. Dr. Fuenzalida
Progesterone	128	128
Δ^6 -progesterone*	147-148	—
Δ^{16} -progesterone	182-188	—
Desoxycorticosterone	140-142	140
Desoxycorticosterone acetate	161	160.5
Dehydrocorticosterone	178-180	—
Testosterone	151-154	155
Testosterone propionate	121-123	121
17-methyl-testosterone	161	157
17-ethyl-testosterone	139	131
17-vinyl-testosterone	127	142
Anhydro-hydroxy-progesterone (17-ethinyl-testosterone)	264-266	266
Dihydrotestosterone	180-181	180
Dihydrotestosterone propionate	121-122	128
17-methyl-dihydrotestosterone	192	191
Pregnane-3,20-dione	118-123	121
Allo-pregnane-3,20-dione	199-200	200
Androstene-3,17-dione	172-174	172
Cholestenone	80.5-82	78-80
Methyl-dehydrocholate	236-239	236
Androsterone	178	178
Androsterone benzoate	178-178.5	178
Androstane-3,17-diol	221-223	221
Androstane-3,17-diol propionate	120-121	120
Δ^5 -androstene-3,17-diol	178-179	166
17-(β)-ethyl- Δ^5 -androstene-3(β),17(α)-diol	198	194.5
Allo-pregnane-3,21-diol-11,20-dione (Kendall's compound H)	172-176	—
Pregnenolone-3-acetate	149	—
Δ^5 -21-acetoxy-pregnene-3-ol-20-one	183	183

* Quantities available were not sufficient to allow for a thorough study.

to the known finding of Deanesly and Parkes (1936) with the estrogenic action of very large quantities of androgens.

Let us now refer to the double bond Δ^4 in ring A of the four afg. steroids. We have tried dihydrotestosterone, a synthetical compound due to the Ruzicka group; its androgenic activity is similar to that of testosterone. Dihydrotestosterone was no less afg. than testosterone (Vera, 1942); results were more constant than with testosterone.

It was but natural that having made the finding with dihydrotestosterone we were eager to examine pregnanediol and allo-pregnadiol lacking the double bond Δ^4 but with a side chain at C₁₇ like progesterone, the most active antifibromatogen; pregnanediol and allo-pregnadiol are void of any known activity. Both these steroids showed no afg. activity, in any case with quantities several times greater than the afg. threshold of progesterone (figs. 67 and 69B; Lipschutz, Bruzzone and Fuenzalida, 1944). There was a doubtful afg. action with quantities 10 to 30 times those of progesterone (table 8).

After this spectacular failure we turned back to compounds with the double bond Δ^4 . Two steroids with a longer side chain at C₁₇ were tried—cholestenedione and methyldehydrocholate. Prolongation of the chain was detrimental. Cholestenedione was not afg. with 160 μg per day, or about 8 times more than with progesterone (Iglesias and Lipschutz 1944). Methyldehydrocholate was still inactive with more than 1000 μg per day (Iglesias, unpubl.).

Chapter 13.

THE QUESTION OF CONCOMITANCY OF ANTIFIBROMATOGENIC AND PHYSIOLOGICAL ACTIONS OF STEROID COMPOUNDS

The quantitative results with the afg. activity of different steroids referred to above called also for a discussion of the question whether there is a concomitancy of the afg. activity and the known physiological actions of these steroid compounds. It may be said beforehand that our findings based on systematic research gave sufficient evidence that it is out of question simply identifying afg. activity with the physiological actions of these steroids already known before (Lipschütz, 1946, 1947).

A. ANTIFIBROMATOGENIC AND PROGESTATIONAL ACTION

Progestational action seemed, at the beginning, to be quantitatively concomitant with the afg. one. Two different groups of facts were in favor of this concept:

First: afg. action of 3-keto-steroids decreases from progesterone to desoxycorticosterone and testosterone and so also their progestational action.

Second: steroids with a very low, or no, progestational action, such as pregnanediol, cholestenone and methyldehydrocholate, revealed no afg. action in quantities several times greater than the afg. threshold of progesterone (tables 8 and 9).

The second of these statements has been studied also with various other steroid compounds. Mention has already been made of steroids with an hydroxyl group at C₃: none of these compounds was afg. unless very large quantities were used. They are not known to have any progestational action, with the possible exception of pregnenolone derivatives whose progestational activity is in any case a very low one. There seemed to be even more, and direct, experimental proof in favor of a concomitancy between progestational and afg. action. We owe to the groups of Butenandt and of Miescher a series of compounds which differ from progesterone by additional double bonds, as for instance Δ^6 and Δ^{16} .

Whereas the Δ^6 compound is progestational the Δ^{16} compound is void

of progestational action with quantities about five times greater than progesterone. We tried Miescher's Δ^{16} compound in our comparative experiments with the afg. action. Quantities of Δ^{16} -progesterone four to five times greater than the afg. threshold of progesterone were unable to prevent estrogen-induced fibroids (figs. 67 and 69B). The above statements are summarized in table 9.

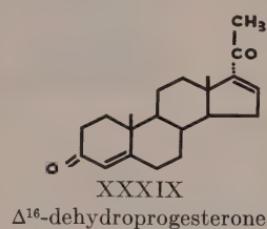
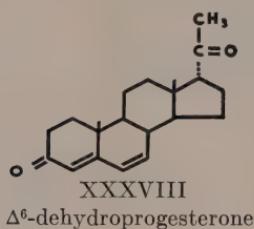


TABLE 9

Steroid compound	Progestational action mg/I.U.*	Anti-fibromatogenic action
		$\mu\text{g per day}^\dagger$
Progesterone	1	15-20
Desoxycorticosterone acetate	10	40-90
Dehydrocorticosterone	[6]	100
Testosterone	140-200	150 (?)
Testosterone propionate	50	130
Progesterone	1	15-20
Δ^{16} -progesterone	100	[160]
Pregnanedione	[50]	[260]
Δ^6 -pregnenolone acetate	40-100	550
21-acetoxy-pregnolone	200	[1240]‡

[] No action.

* mg/I.U.—quantity injected into rabbits, according to Selye and Masson, 1943.

† Absorbed from subcutaneously implanted pellets.

? Seemingly active at lower levels also but not constant; see ch. 11, C.

‡ Slight action.

The comparative behavior of progesterone on one hand, and of the non-progestational pregnanediol and Δ^{16} -progesterone on the other hand, has been studied also in experiments with estrogen-induced thickening of the conjunctive capsule which envelops a metallic pellet in the abdominal cavity (Bruzzone, Schwarz and Lipschutz, 1944; see also p. 49). Pellets of estradiol were implanted beneath the skin and so also pellets of the four 3-keto steroids mentioned. Forty days later the metallic pellet was introduced into the abdominal cavity. The animals were necropsied ninety

days after implantation of the steroid pellets. The results are given in the adjoining table 10 and in figs. 72 to 77. The table and figures show that the estrogen-induced thickening of the capsule was prevented in most of the animals with progesterone but not with the other three steroids. When reaching this phase of our work we were almost convinced that there is a concomitancy between progestational and afg. action. But new experiments have shown that both these actions cannot be identified.

TABLE 10

Fibromatogenic steroid	Additional 3-keto-steroid	Number of animals in the experiment	With tumoral thickening of the capsule		Quantity of 3-keto-steroid per day
			No. of animals	Per cent of animals	
α -estradiol	0	7	6	90	0
α -estradiol	progesterone	14	2	15	8-24
α -estradiol	pregnanedione	10	8	80	10-62
α -estradiol	allo-pregnandione	6	6	100	6-30
α -estradiol	Δ^{16} -progesterone	9	9	100	10-78

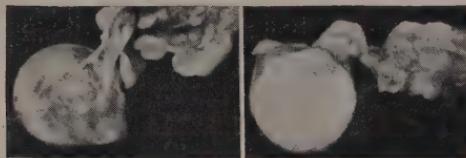


FIG. 72. Behavior of a metallic pellet in the abdominal cavity of the guinea pig. Necropsy at 309 days. The pellet became attached to the epiploon and is surrounded by a thin capsule. $\times 0.6$. (male G.p. XXIX.28).

Progestational action of androgens is enhanced by substituting in testosterone the hydrogen at C₁₇ especially with a side chain of two carbons (Klein and Parkes, 1937). Will the afg. activity of testosterone also be enhanced by such a substitution at C₁₇? The following five compounds were examined: ethinyl-, methyl-, ethyl-, vinyl-testosterone, and methyl-

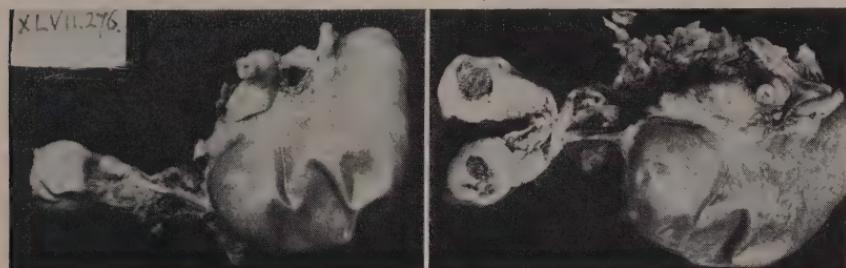
dihydrotestosterone (Lipschutz 1946, 1947; Lipschutz, Iglesias et al., 1948a).

The general result of these experiments was not in favor of concomitancy of progestational and afg. actions: the afg. activity of these androgens was



Progest. 13 μ g

FIG. 73. Protective action of progesterone against estrogen-induced thickening of the capsule of metallic pellet attached to the epiploon. Dorsal and ventral view. 37 μ g of estrad. and 13 μ g of progesterone daily, 90 days. The metallic pellet was since 49 days in the abdominal cavity; the borders of the capsule are clear cut. Size 1.3. (fem. G.p. XLVII.252).



Pregnanedione 29 μ g

FIG. 74. Non-protective behavior of pregnanedione. 43 μ g of estrad. and 29 μ g of pregnanedione daily, subcut. pellet, 91 days. (fem. G.p. XLVII.276). The metallic pellet is attached to the epiploon; since 49 days in the body. A. Tumoral thickening of the capsule. B. Tumor opened to show the pellet whose borders became irregular. Size 0.7.

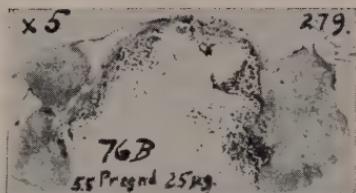


Δ^{16} -Dehydroprog. 42 μ g

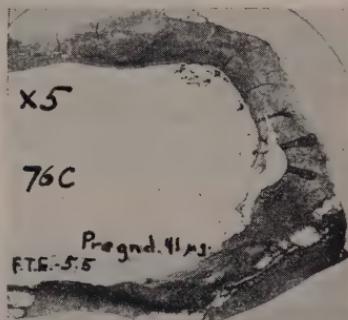
FIG. 75. Non-protective behavior of Δ^{16} -dehydroprogesterone. Dorsal view of metallic pellet attached to the epiploon. 49 μ g of estrad. and 42 μ g of Δ^{16} -dehydroprogesterone daily, 91 days. Thick tumoral masses around the metallic pellet. 50 days in the abdominal cavity (fem. G.p. XLVII.222).



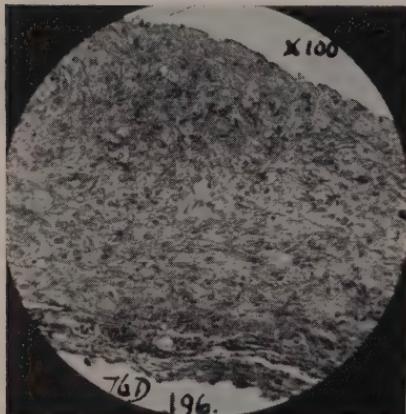
Pregnanned. 14 μ g



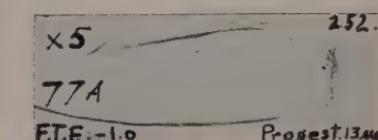
Pregnanned. 25 μ g



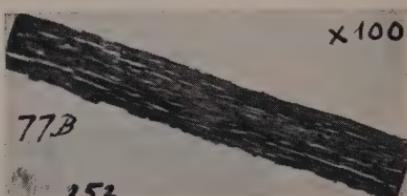
Pregnanned. 41 μ g



Allo-pregnanned. 27 μ g.



Progest. 13 μ g



Progest. 13 μ g

FIG. 76. Capsule of metallic pellet in animals receiving simultaneously estradiol and different 3-keto-steroids; since 49 to 50 days in the abdominal cavity. To be compared to fig. 77A: A. 41 μ g of estrad. and 14 μ g of pregnanediol daily, 91 days. \times 5. (fem. G.p. XLVII.270). B. 46 μ g of estrad. and 25 μ g of pregnanediol daily, 91 days. \times 5. (fem. G.p. 279). C. 42 μ g of estrad. and 41 μ g of pregnanediol daily, 92 days. \times 5. (fem. G.p. XLVII.287). To be compared to fig. 77B: D. 26 μ g of estrad. and 27 μ g of allo-pregnannedione daily, 91 days. \times 100. (fem. G.p. XLVII.196).

FIG. 77. Capsule surrounding the metallic pellet in an animal receiving simultaneously estrad. and progesterone. Same as fig. 73. A. \times 5. B. \times 100. (XLVII.252).

not enhanced, and sometimes even diminished by a substitution by which their progestational action is enhanced.

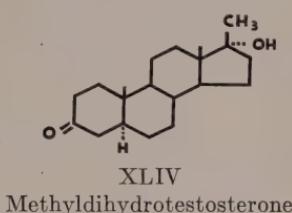
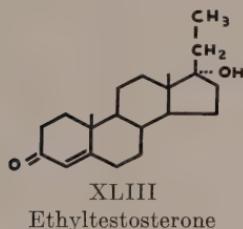
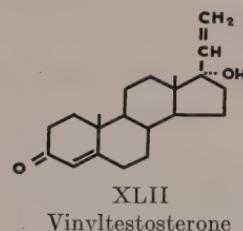
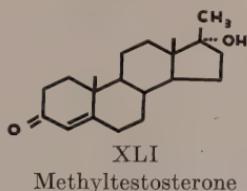
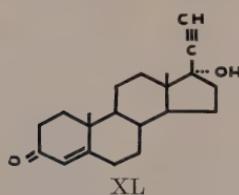


TABLE 11

Steroid compound	Progestational action	Antifibromatogenic action
	mg/I.U.*	μg per day†
Testosterone.....	140-200	>150?
Testosterone propionate.....	50	>130
17-methyl-testosterone.....	30	150
17-vinyl-testosterone.....	<20	[>350?]
17-ethyl-testosterone.....	—	[>210?]
17-ethinyl-testosterone.....	10	>190
Dihydrotestosterone.....	—	>110
17-methyl-dihydrotestosterone.....	—‡	>110
Δ^4 -Androstene-3-17-dione.....	140-200	[570]

* mg/I.U.—quantity injected into rabbits, according to Selye and Masson, 1943.

[] No action.

? Seemingly active at lower levels also, but not constant.

† Absorbed from subcutaneously implanted pellets.

‡ Similar to methyl-testosterone, according to Klein and Parkes, 1937.

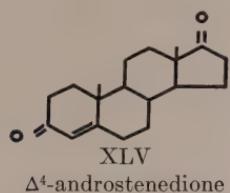
Let us take first ethinyl-testosterone, the well known steroid compound due to the Ruzicka group and to German workers. Under the name of anhydro-hydroxy-progesterone it has attracted great interest among clinicians on account of its having progestational action and being equally

active by mouth as by injection, whereas progesterone when given by mouth loses greatly in activity. Ethinyltestosterone prevents in the guinea pig estrogen-induced fibroids; but its afg. activity is many times smaller than that of progesterone. Its afg. threshold quantity is about fifteen times that of progesterone; since its progestational activity is only about a tenth that of progesterone the result might seem in favor of concomitancy of the two activities. But when comparing ethinyltestosterone to testosterone it becomes evident that such an interpretation would be erroneous: the ethinyl derivative though more progestational than testosterone is certainly not more afg. By enhancing progestational activity by a substitution at C₁₇ one does not enhance afg. activity. It was on account of these statements with ethinyl-testosterone that experiments with other testosterone derivatives were undertaken. Though results with some of these compounds, especially ethyl- and vinyl-testosterone are not definite (see table 11) they are sufficient to show conclusively that afg. activity cannot be identified with the progestational one. This is corroborated by other statements. The two pregnenolones have only a very slight afg. activity (table 9) but they are as progestational as is the afg. testosterone (see Selye and Masson 1943; Nos. 25 and 26 of their table 1). This refers also to androstenedione (see section B).

B. ANTIFIBROMATOGENIC AND MASCULINIZING ACTION

Neither was there concomitancy of afg. activity with the masculinizing one. Results are summarized in table 12.

Transformation of the clitoris into an hypospadic penis takes place in the female guinea pig under the influence of testicular endocrine action, as I found about thirty-five years ago in one of Steinach's experimental animals (summary Lipschutz, 1924). Later on, in experiments with the injection of chemically pure androgens, we effected the transformation to a degree visible to the naked eye in 4 to 7 days (Ruz, 1939). We never saw this masculinizing action with quantities of progesterone or desoxycorticosterone acetate many times greater than the afg. threshold. On the contrary, with testosterone or dihydrotestosterone the clitoris transforms into an hypospadic penis various mm long with quantities still unable to prevent the estrogen-induced fibroids. This finding has been corroborated with the 17-methylderivatives of testosterone and dihydrotestosterone, with ethyl- or vinyltestosterone. On the other hand, ethinyltestosterone was unable to masculinize with 300 µg per day though there was afg. action already at lower levels. Results with Δ⁴-androstene-3-17-dione were especially striking. With 570 µg per day the hypospadic penis was 5 mm long whereas this quantity was not yet afg.; transformation of the clitoris into the hypospadic penis begins already with 230 µg of androstenedione per day (Iglesias and Lipschutz 1944).



Ample use has been made of testosterone in clinical work with the special purpose of antagonizing atypical growth of cells dependent on estrogen—in any case under normal circumstances. Clinicians have claimed success in cystic glandular hyperplasia and other pathological conditions. On the basis of our work with androgens one may question whether this antagonizing faculty of testosterone is really due to its being an androgen. This

TABLE 12

Steroid compound	Masculinizing action*		Anti-fibromatogenic action μg per day†
	μg per day†	Length of clitoris mm	
Progesterone.....	300	0	15-20
Desoxycorticosterone acetate.....	200	0	40-90
Testosterone.....	15-20	3	>150?
17-methyl-testosterone.....	47	2	150
17-vinyl-testosterone.....	350	2	[>350?]
17-ethyl-testosterone.....	210	1	[>210?]
17-ethynodiol-estradiol.....	300	0	>190
Dihydrotestosterone.....	40	4	>110
17-methyl-dihydrotestosterone.....	74	2	>110
Androstenedione.....	230-570	5	[570]

[] No action.

* Unpublished results.

† Absorbed from subcutaneously implanted pellets.

conclusion may seem at first sophisticated. But it is coincident with the conclusion reached in work with the afg. action of progesterone which neither is due to its being progestational.

C. ANTIFIBROMATOGENIC AND CORTICAL ACTION

We have referred to two afg. cortical steroids: desoxycorticosterone and dehydrocorticosterone. But afg. activity is not concomitant with the "corticoid" action, judged by the faculty to maintain alive the suprarenalecotomized animal. The experimental statements are summarized in

table 13 (Bruzzone, Borel and Schwarz, 1946; Borel, 1945; Bruzzone and López, 1948; López, 1947).

About 175 μg of desoxycorticosterone acetate, or more, are necessary for maintaining alive the suprarenalectomized guinea pig (Bruzzone et al., 1946). But the afg. threshold quantity of desoxycorticosterone acetate is certainly less than 100 μg per day. Desoxycorticosterone can be replaced as to corticoid action by 21-acetoxypregnolone (Selye, 1941a; Segaloff and Nelson, 1942; Bruzzone et al., 1946); the suprarenalectomized guinea pig survives for many months with quantities of this compound about 5 times larger than those of the cortical hormone. But these quantities of acetoxypregnolone are yet insufficient to prevent estrogen-induced fibroids. On the contrary, pregnenolone acetate whose corticoid activity is

TABLE 13

Steroid compound	Corticoid action		Anti-fibromatogenic action $\mu\text{g per day}^\dagger$
	$\mu\text{g per day}$	Survival > 9 days per cent	
Progesterone.....	—	—	15-20
Desoxycorticosterone acetate.....	175-525	80	40-90
21-Acetoxypregnolone.....	700-1250	80	[1240]
Pregnenolone-3-acetate.....	733-2625	28	550-1670

[] No action, or slight action.

† Absorbed from subcutaneously implanted pellets.

considerably smaller than that of 21-acetoxypregnolone is more afg. than the latter (Iglesias and Bruzzone, 1948).

D. ANTIFIBROMATOGENIC AND ANTIESTROGENIC ACTION

A wealth of experimental evidence has been given above in favor of the statement that afg. action cannot be understood as concomitancy with, or sequence to, known physiological faculties of steroids as progestational, androgenic are cortical. Afg. action is a common activity denominator of these steroids. There seems to be, in any case at the first glance, also an other common activity-denominator—their antiestrogenic action.

Let us take first the behavior of the vagina. When pellets of estradiol and progesterone are implanted simultaneously into a castrated female guinea pig the genital opening becomes viable as with estradiol alone. But about 2 to 4 weeks afterwards it closes again and remains so till the end of the experiment. Closure with progesterone and also desoxycorticosterone is so definite and prolonged that in similar experiments the uterus may become cystically enlarged (fig. 78). With testosterone closure is also ob-

tained but with less constancy; the vagina was open, or closure was not perfect, in animals in which there was an unmistakable afg. action.

An other antiestrogenic action common to these steroids was that on the uterus. With afg. quantities of the different steroids given simultaneously with the estrogen increase of the uterine weight was less than with estrogen alone. Uterine weight varies greatly with the prolonged administration of estrogens. But as shown in table 14 there was no doubt about the faculty of these steroids to counteract the hysterotrophic action of estrogen.



FIG. 78. Cystic enlargement of uterine horns in animal with permanent closure of the genital opening induced by progesterone. 40 µg of estrad. daily, subcut. pellet of estrad. diprop., and 39 µg of progesterone daily every 10 days from subcut. pellet (discontinuous action, see fig. 84). Duration of the experiment 106 days. A. No uterine tumors. Above—cystic enlargement. Nat. size. B. Section of enlarged upper part. Digitations of the endometrium. Muscular wall appears thin on account of distension. $\times 5$. C. Section through narrow part. Note hypertrophy of the ventral muscular ridge which occurs under the influence of the estrogen (comp. fig. 1B and 66). $\times 5$. (LXI.3).

Opening of the vaginal entrance and increase of uterine weight—the latter being due predominantly to the increase of the myometrium—are but normal effects of estrogenic action. With the continuous administration of the estrogen the condition becomes abnormal only in so far as the entrance remains open continuously and the uterine weight attains a level higher than normal. But *toxic* effects also were produced. There was first cystic glandular hyperplasia and the adenomatous proliferation of the uterine glands (chapt. 6, E). There was the abnormal vascularization of the uterine submucosa with rupturing vessels, extravasation and formation of pigment in the submucosa. There was uterine bleeding which does not

occur in the normal animal. There was also a more or less ample necrosis of the endometrium and necrosis of the myometrium. In these cases of necrosis there was also pyometra with cellular invasion of the myometrium and in some cases pus on the uterine serosa (p. 118). How did all these estrogen-induced toxic effects behave when afg. quantities of different steroids were administered simultaneously with the estrogen? Let us take first uterine bleeding, which occurred in about 30 per cent of animals receiving estrogen

TABLE 14

All animals receiving α -estradiol and different antifibromatogenic steroids administered by subcutaneously implanted pellets

Antifibromatogenic steroid	Afg. steroid per day	Dura-tion	Number of animals Total	F.T.E. Average	Uterine weight range	Uterine weight average	Number of animals with uterine necrosis	Number of animals with uterine bleeding
0 (XXVII)*	0 μg	2 months	29	3.0	2.1-6.2	3.7	8	8
Progesterone (II Tab.)	300-1000	2	14	0.5	1.5-2.4	1.9	0	0
Desoxycorticosterone acetate (XXIII)	125	2	17	0.5	1.0-3.3	1.9	0	0
Testosterone propionate (XXVI)	180	2	18	1.8	1.2-4.2	2.7	0	2
0 (XLVII)	0	3	15	5.1	3.0-12.0	5.7	3	4
Progesterone (XLVII)	13-24	3	14	1.4	1.7-5.1	3.0	3†	0

* Number of series.

† The quantities used were near the antifibromatogenic threshold of progesterone.

alone (table 14). With the additional administration of progesterone or desoxycorticosterone the vaginal entrance is almost always closed in such a manner that uterine bleeding even if occurring could not have been stated. But microscopical examination revealed that with these two afg. steroids there was no abnormal vascularization of the uterine submucosa, no extravasation and no accumulation of pigment; there was no necrosis of the myometrium or endometrium. On the contrary, with quantities of testosterone as used in these experiments (table 14) uterine bleeding may still

occur as also necrosis may be found with small quantities of progesterone near to the afg. threshold. One must suppose that the non-coincidence between afg. action, on one hand, and antagonizing of different toxic actions of the estrogen on the uterus on the other hand, was due to a difference of thresholds for the different receptive territories or tissues. This is especially well exemplified by the behavior of the uterine epithelium which has been examined in 76 out of the 78 animals of the upper part of table 14. Cystic glandular hyperplasia and adenomatous proliferation were with the additional action of the antifibromatogenic steroids much less pronounced than with estrogen alone. Likewise the estrogen-induced increase of the alkaline phosphatase in the uterine mucosa (see p. 117) has been counteracted with afg. steroids as progesterone, dihydrotestosterone or methyltestosterone (Fuenzalida, 1949; Pinto, 1946; Nahmías, 1947; Díaz, 1947; Tenorio, 1948). But even with large quantities of progesterone or desoxycorticosterone prevention of cystic glandular hyperplasia was not complete. Occasionally even metaplasia of the endometrium was still present. All this applies especially to testosterone (see also p. 168).

These data though sometimes conflicting, would not be definitely contrary to the concept of a concomitancy between afg. and antiestrogenic actions. But there were two other experimental statements which gave seemingly convincing evidence that it would be out of question simply identifying afg. with antiestrogenic actions.

First: Progesterone, desoxycorticosterone, testosterone which prevent estrogen-induced abdominal fibroids, i.e. exert an antagonistic action against the estrogen on the peritoneal cover, do not counteract at all the morphogenic action of the estrogen on the nipples and the mammary glands (Lipschutz and Vargas, 1941c; Lipschutz and Zañartu, 1942; Zañartu, 1943; González, 1943). They were, especially with desoxycorticosterone, even more developed than with estrogen alone. It was often the same with other additional afg. steroids. A stimulative action of androgens and desoxycorticosterone on the mammary gland has been repeatedly reported by workers in former and subsequent years (Von Wattenwyl 1944, p. 181).

Second: Progesterone which is the most potent afg. and antiestrogenic steroid in the guinea pig is unable to counteract the estrogen-induced tumoral growth of the hypophysis in the rat (Lipschutz; see Riesco and Anfossi, 1948; Anfossi, 1947). To take an example: even with as much as 194 µg of progesterone per day, as against 11 µg of stilbestrol, an hypophysial weight of 163(!) mg was attained in the course of 4 months. One may indeed ask whether these results were due to having used stilbestrol instead of natural estrogens since Smith and Smith (1944) have reported that progesterone which in the rat antagonizes the increase of hypophysial

weight induced with estrone in the course of 5 days, is not capable to counteract a similar increase when induced with stilbestrol. But on the other hand, Albert and Selye (1942) were not able to obtain in the rat clear cut results when trying to antagonize with progesterone the increase

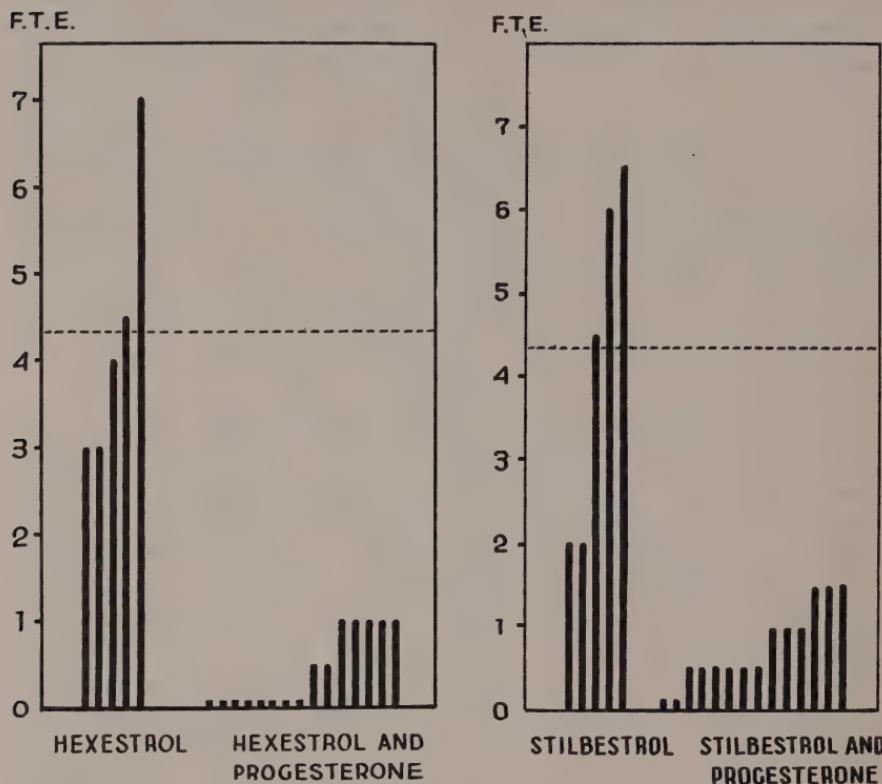


FIG. 79. Afg. action of progesterone in experiments with stilbestrol and hexestrol (fem. G.p. LVIII). A. 6 to 20 µg of stilbestrol per day in the control group; 11 to 12 µg of stilbestrol and 120 to 305 µg of progesterone per day in the stilbestrol-progesterone group. B. 3 to 10 µg of hexestrol in the control group; 8 to 13 µg of hexestrol and of 122 to 261 µg of progesterone in the hexestrol-progesterone group. 90 days. No animal receiving progesterone attained the minimum of the hexestrol or stilbestrol group.

of hypophysial weight induced with estradiol in the course of 14 days, even with as much as 10 mg of progesterone as against 300 µg of estradiol per day. There is also the fact that progesterone promptly antagonizes fibromatogenic action of stilbestrol and hexestrol in the guinea pig (ch. 14; fig. 79). In the work of Hertz et al. (1947) with the inhibition of estrogen-

induced growth of the oviducts in chicks with progesterone (ch. 11, B) stilbestrol was used; progesterone was so effective against 4 mg of stilbestrol as against 1 mg of estradiol benzoate.

The above statements demonstrate that an afg. steroid which is effective as an antagonist of estrogenic actions in certain territories may be void of any such effect in other territories. It is not possible to presume an afg. activity of a steroid from what is known on its antiestrogenic actions or vice versa. Non-concomitancy of afg. activity with other physiological activities as pregestational, masculinizing and cortical, and so also non-concomitancy of afg. and antiestrogenic activity suggest that afg. action is independent of any other known action of the steroids. *On a purely experimental basis one may conclude that afg. action exemplifies a certain type of antitumoral action per se.*

Our conclusion is coincident with existing knowledge on the inhibition of estrogen-induced growth of the anterior lobe of the hypophysis with androgens and related compounds. It has been found in older work of ours (Lipschutz, 1936c, 1942d) that in the rat, where the weight of the anterior lobe differs in the two sexes—2.5 mg in the male, 4 mg in the female p. 100 g body weight—, it weighs 4 mg in both when castrated; that is to say it has been “neutralized”. After castration also “neutralization” as to the luteinizing faculty of the hypophysis takes place when examined by administration to infantile females. According to the ample work of Wolfe and his associates the testicular control of the hypophysial weight is due to testosterone; the latter counteracts the pituitary changes in old male rats (Wolfe, 1941) and antagonizes, in experiments of short duration (15 days), the estrogen-induced increase of the hypophysial weight (Wolfe and Hamilton, 1937, 1939). Testosterone antagonizes also the tumorigenic action of estrogen on the anterior lobe in mice (Gardner, 1947c). This faculty is shared by methyltestosterone and other androgens (Albert and Selye, 1942), even then when stilbestrol instead of natural estrogen is used (Haour, 1948). But the remarkable statement also has been made that the faculty of testosterone to control the hypophysial weight is not a sequel of its androgenic activity: cis-testosterone which is void of androgenic activity also antagonizes the estrogen-induced hypophyseal increase (Haour and Selye, 1948). Its antagonizing faculty on the hypophysis was even more pronounced than that of various androgens and inferior only to that of Δ^4 -androstenedione, a steroid which in our work was void of antifibromatogenic activity (see table 11).

These comparative statements substantiate our conclusion that antitumoral actions of steroids cannot be considered simply as a sequel of known physiological actions inherent to these compounds. The problem

is also of importance in the hormonal treatment of prostatic carcinoma. Is the therapeutical action of stilbestrol due to its being estrogenic? The group of Lacassagne (Berger and Buu-Hoi, 1947) has tried in prostatic carcinoma phenylbromethylene (Y59) which in the rat is 100 times less estrogenic than stilbestrol but its action is a very protracted one (Lacassagne et al., 1946). Quantities not superior to, or even smaller than, those of stilbestrol were used; notwithstanding that there was seemingly clinical success.

Chapter 14.

THE MODE OF ACTION OF ANTIFIBROMATOGENIC STEROIDS

How is the preventive action of progesterone and other steroids against estrogen-induced fibroids effected? The question is all the more intricate as we do not even know where and how estrogen is acting when producing for instance its physiological effect on the vaginal mucosa. Epidermization of the explanted vaginal mucosa was not obtained by adding estrogen (Emmens and Ludford, 1940).

According to our original concept of about 25 years ago antagonistic action was due to desensibilization of the receptive tissue by the hormone of the opposite sex; antagonism was supposed to be a localized, or "territorial," action. This supposition was based on experimental findings with ovarian grafts, and it was subsequently substantiated by various workers with the administration of hormones, especially by Courrier and his associates (p. 126). Can this concept be applied to the prevention of estrogen-induced fibroids by steroids? The question is of relevance; it is likewise the question of the site of action of estrogen in the hormonal treatment of prostatic cancer in man, or of cancer of the breast with androgens and estrogens.

There is no direct proof of a peripheral, or territorial, antagonistic interplay of steroids. Recently the question has been studied by Bruzzone and Toro in what seemed to be a clear cut experimental setting but with negative results. Fibromatogenic quantities of estrogen (estradiol, stilbestrol) were absorbed from a subcutaneously implanted pellet whereas a pellet containing 40 per cent of progesterone was implanted into the spleen (fig. 80). Estrogen was to circulate in the whole body and to reach also the splenic serosa; progesterone being inactivated in the liver (p. 205) was to circulate and act in the spleen only. The experiments lasted 4 to 5 months; fibroids were produced on the splenic serosa as without the local action of progesterone (Toro, 1947). Under the given experimental conditions there was no antagonistic interplay of the two steroid on the splenic serosa. There being no direct proof in favor of an antagonistic interplay in the receptive tissue in *all* instances (for proofs in other instances see p. 126)

the authorities writing on the antagonism of sex hormones for many years strongly opposed our concept. They assumed that the antagonizing hormone, say the estrogen, interferes primarily in the gonadotropic function of the hypophysis so that the gonad ceases producing its specific hormone, androgen. We have already seen that this assumption is contrary to existing experimental evidence; testosterone antagonizes estrogen also in the *castrated* rat (p. 126)! It must be strongly emphasized that the hypophysial gonadotropic mechanism has nothing to do with the afg. action of steroids: the afg. action of progesterone and other 3-keto-steroids is not dependent

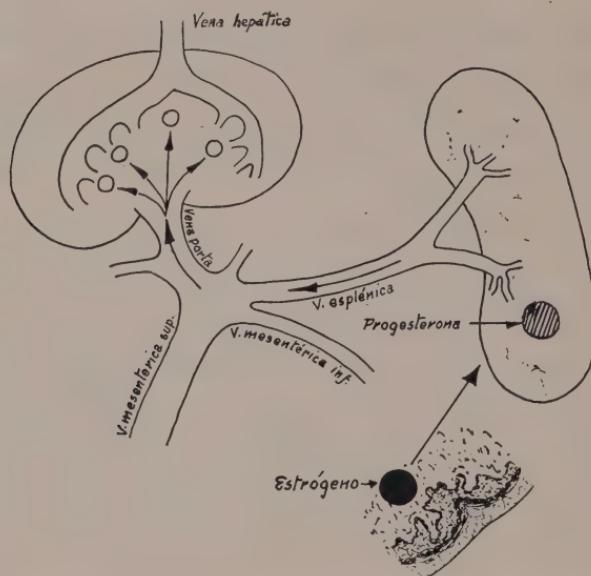


FIG. 80. Simultaneous action of subcut. implanted pellet of estrogen, and of pellet of progesterone implanted into the spleen. The estrogen reaches the spleen by the general circulation; progesterone circulates in the spleen only being inactivated in its passage through the liver. There was no inhibition of tumors on the spleen (device of Bruzzone; see Toro, 1947).

on the presence of the ovary, i.e. afg. action of progesterone is not effected by the intermediance of hypophysial gonadotropic hormones. Our work on the preventive action of steroids was done with the simultaneous administration of estrogen and afg. steroids to *castrated* animals, that is to say under experimental conditions in which there is no place for hypophysial gonadotropic factors.

One may ask whether afg. steroids interfere somehow in the enzymatic mechanism of the liver by which ovarian estrogens are inactivated or transformed into less active urinary estrogens. This is not very likely. Though the behavior of different estrogens in the liver varies so greatly they can

be all antagonized by progesterone. Esterified estradiol is more resistant against inactivation in the liver than the free hormone as shown by injection in the spleen of the rat (Segaloff and Nelson, 1941; Segaloff, 1943), or by implanting pellets of free or esterified estradiol (17-caprylate, dipropionate) into the spleen or liver (Lipschutz and L. Acuña, 1943; Lipschutz and Carrasco, 1943; L. Acuña 1942; Carrasco 1942). Notwithstanding that, progesterone was quantitatively as preventive against the fibromatogenic action of these esters as against the free hormone (Lipschutz and Grismali 1944; Grismali 1942). Artificial estrogens as stilbestrol and hexestrol also are more resistant than natural ones as has been shown by experiments *in vitro* (Zondek et al., 1943), with intrasplenic injections (Segaloff, 1944), or with the absorption of fibromatogenic quantities of stilbestrol and hexestrol from pellets implanted into liver or spleen (Lipschutz, Quintana and Bruzzone, 1944; Quintana, 1943; fig. 98). But here again, greater resistance against inactivation in the liver does not protect against the antagonistic action of progesterone; the latter prevents also fibroids induced by artificial estrogens (Bruzzone, 1949; Peña, 1943; fig. 79). The quantity of progesterone necessary in the latter case though somewhat greater than with estradiol was not far from that (Bruzzone 1949).

In recent work on the influence of progesterone on the estrogenic threshold in rats Segaloff (1947) reached the conclusion that progesterone does not enhance, but even counteracts, inactivation of estradiol in the liver. Thus, for the moment, nothing seems contrary to the tentative assumption that the antagonistic action of progesterone is due to desensibilization of the receptive tissue.

Various authorities have emphasized that there may be also other ways in which estrogenic action is counteracted by progesterone the latter for instance enhancing conversion of estradiol into estriol, or the excretion of estradiol (Pincus and Zahl, 1937; Smith and Smith 1946). This would, indeed, not be contrary to a simultaneous desensibilizing action on the receptive tissue.

Chapter 15.

HORMONAL TREATMENT OF ESTROGEN-INDUCED TUMORAL GROWTH IN EXPERIMENTAL ANIMALS

A. GENERAL

Hormonal treatment has been tried in human beings in different cases of atypical proliferation, both conjunctive and epithelial: against uterine fibroids and benign prostatic hyperplasia, against cystic glandular hyperplasia of the uterus, against cancer of the mammary glands and against prostatic cancer. Progesterone, testosterone and estrogens have been made use of; the estrogenic steroid has been replaced also by stilbestrol and other artificial estrogens. It cannot be our task to discuss experimental and clinical work in their many details. Experimental work both preventive and therapeutical, and clinical work done in this field since 1936 are summarized in table 15.

Some striking aspects of clinical application may be emphasized. Loeser (1938), Greenblatt (1943; 1944) and Vargas et al. (1945) have claimed success in treatment of *uterine fibroids* with the subcutaneous implantation of pellets of testosterone propionate; they reported unanimously a considerable decrease in size of the palpable tumors though the latter never disappeared. Goodman (1946) reported shrinkage of uterine fibroids with subcutaneously injected progesterone. Segaloff et al. (1946) found by bimanual examination a definite reduction in the size of the fibromyoma in one patient but roentgenograms with contrast medium showed no such change (Segaloff et al. 1949).^{*} Testosterone propionate has been used against *mammary cancer* though sometimes with conflicting results (Fels, 1944b; Prudente, 1945; Adair and Herrmann, 1946; Herrmann and Adair, 1946; Adair, 1947; Escher et al., 1947). Contradictory as it may seem at the first glance, estrogens especially stilbestrol, sometimes triphenylchlorethylene, have been used against the same neoplastic disease in aged women (Binnie, 1944; Lancet, 1944; Haddow et al., 1944; Nathanson, 1947; Walpole and Paterson, 1949).

The treatment of *prostatic cancer* with estrogens introduced several years ago by Huggins (1946, 1947, 1949) is amply known. This treatment

* See also discussion of these results at the end of section C.

TABLE 15*

Preventive and therapeutical actions of steroids in experimental and spontaneous atypical proliferation or neoplastic growth

Type of tumor, or atypical proliferation	Type of experiment	Antitumorigenic steroid used (or synthetic compound of an action similar to that of a steroid)	Author
<i>Estrogen-induced:</i>			
Metaplasia of cervical mucosa in Rhesus	Preventive	Extract with pregestational action	Hisaw & Lendrum (1936)
Hyperplasia of prostatic stroma and metaplasia of utricular mucosa in Rhesus	Preventive and therapeutic	Androstanediol and testosterone propionate; progesterone	Zuckerman & Parkes (1936)
Hyperplasia of prostatic stroma and metaplasia of utricular mucosa in the dog and mouse	Preventive and therapeutic	Stimulating the production of testicular hormone by gonadotrophic hormone	De Jongh et al. (1938)
Hyperplasia of prostatic stroma and metaplasia etc. in the rat and mouse	Preventive	"Testicular hormone," Testosterone, progesterone, and combination of both	Korenchevsky & Dennison (1935); Rusch (1937); Harsh et al. (1939); De Jongh et al. (1939)
Metaplasia of the uterine epithelium in the rat	Preventive	Progesterone	Korenchevsky & Hall (1938, 1940)
Mammary tumor, able to produce metastases and sometimes transplantable in the rat	Therapeutic	Progesterone	Noble & Collip (1941a)
Uterine and abdominal fibroids in the guinea pig	Preventive	Progesterone; desoxycorticosterone, acetate or free; dehydrocorticosterone; testosterone propionate or free; dihydrotestosterone, propionate or free; ethynodiol; methyl-dihydrotestosterone	Lipschutz, Vargas, Iglesias Bruzzone et al. (1938-1946)
Uterine and abdominal fibroids in the guinea pig	Preventive	Progesterone; desoxycorticosterone acetate; testosterone propionate	Von Wattenwyl (1944)
Uterine and abdominal fibroids in the guinea pig	Therapeutic	Progesterone	Lipschutz & Maass (1944); Lipschutz & Schwarz (1944)
Adenomatous proliferation of uterine mucosa in the guinea pig	Preventive	Progesterone; desoxycorticosterone acetate; testosterone propionate	Lipschutz, Vargas & Ruz (1939); Lipschutz et al. (mostly unpublished); Von Wattenwyl (1944)
Utricular fibromyoepithelioma in the guinea pig	Preventive (therapeutic so far not successful)	Progesterone; desoxycorticosterone acetate; testosterone propionate	Lipschutz, Yanine et al. (1945)
Metaplasia of glandular cells of mammary fibroadenoma in the guinea pig	Preventive	Testosterone propionate; not successful	Lipschutz (unpublished)
<i>Spontaneous tumors in animals:</i>			
Mammary adenocarcinoma in the mouse	Preventive	Testosterone acetate; not successful	Lacassagne (1937c)
	Preventive	Testosterone propionate; successful	Lacassagne & Raynaud (1939); Nathanson & Andervont (1939); Loeser (1941); Heiman (1944)

TABLE 15*—Continued

Preventive and therapeutical actions of steroids in experimental and spontaneous atypical proliferation or neoplastic growth

Type of tumor, or atypical proliferation	Type of experiment	Antitumorigenic steroid used (or synthetic compound of an action similar to that of a steroid)	Author
	Preventive & therapeutic, the latter not successful	Testosterone propionate	Jones (1941)
	Preventive	Testosterone propionate and progesterone	Heiman (1945)
	Preventive	Progesterone; not successful (Marsh-Buffalo strain)	Bischoff & Rupp (1946)
	Preventive	Progesterone; not successful (C3H strain)	Burrows & Hoch-Ligeti (1946)
Benign mammary adenofibroma in the rat	Preventive and therapeutic	Testosterone propionate and progesterone	Heiman (1943)
Transplantable malignant lymphatic tumor of thoracic cavity in mouse	Preventive and therapeutic	Kendall's compound E: 11-dehydro-17-hydroxycorticosterone	Heilman & Kendall (1944)
Leucemia in the mouse	Preventive	Testosterone propionate	Murphy (1944)
Lymphoid tumors in the mouse	Preventive	Testosterone propionate	Gardner (1944)
Transplantable leucemia in the rat	Preventive	Desoxycorticosterone acetate; cortical extract, total; adrenotropic hormone of the hypophysis	Murphy and Sturm (1944)
Sarcoma 37 in the mouse	Preventive	Adrenal extracts†	Diller et al. (1948; refer to former work with cortical extracts).
<i>Spontaneous tumors in man:</i>			
Mammary carcinoma	Therapeutic	Testosterone (in general propionate)	Many authors (Loeser, 1941; Fels, 1944b, 1948; Prudente, 1945; Adair & Herrmann, 1946; Nathanson et al. 1947; Guggisberg, 1948)
Mammary carcinoma	Therapeutic	Diethylstilbestrol	Binnie (1944); Lancet (1944); Nathanson et al. (1947)
Mammary carcinoma	Therapeutic	Triphenylchlorethylene	Haddow et al. (1944); Nathanson (1947b)
Mammary carcinoma	Therapeutic	Diethylstilbestrol; dienestrol; M 2613	Walpole & Paterson (1949)
Prostatic carcinoma	Therapeutic	Diethylstilbestrol	Huggins & many others (since 1942; 1947, 1949)
Prostatic carcinoma	Therapeutic	Triphenylbromomethylene	Berger and Buu-Hoi (1947)
Uterine fibromyoma	Therapeutic	Testosterone propionate	Loeser (1938); Greenblatt (1943, 1944); Vargas, Vargas & Ossandon (1945)
Uterine fibromyoma	Therapeutic	Progesterone	Goodman (1946)
Uterine fibromyoma	Therapeutic	Progesterone; doubtful results	Segaloff et al. (1946, 1949)
Lymphoid tumors	Therapeutic	"Cortisone" (Kendall's comp. E)	Pearson et al. (1949)

* The list is not complete. For references see also Greenblatt (1944, p. 63), Von Wattenwyl (1944, p. 177-201), Nathanson (1947a, p. 165-166).

† There were also experiments with testis extracts by which growth of a transplantable lymphosarcoma in mice was retarded (Arensen et al., 1949). But the factor was of unknown nature and seemingly not related to any steroid.

has been successful in numerous cases though there were also cases of relapse (Thompson, 1948¹). It is generally accepted that the beneficial effect of estrogen in prostatic cancer is due to a suppression of the release of the gonadotrophic hormones by the hypophysis. But the therapeutical action of estrogens in prostatic cancer, or of androgens in mammary cancer, can be more reasonably explained by the antagonistic peripheral play of these hormones, since the afg. action of steroids becomes manifest also in *castrated* animals. A different way of action may be in play when *estrogen* is made use of in *mammary cancer*; the necrotizing actions of estrogen on receptive tissues when the hormone is administered in sufficient quantities and for a sufficient length of time may be again mentioned here (see pp. 118, 151). There are indeed experimental observations which might tentatively suggest also another mode of action. Eisen (1941) working with a transplantable adenocarcinoma in the rat able to metastasize, has found that some degree of inhibition of tumor growth is obtained by the administration of estrogen by which lactation is induced; the supposition is made that in this way potentially proliferating tumoral tissue is transformed into functioning mammary tissue. What has been said above about suppressing by an adequate stimulus one of the "competences" of a tissue and inaugurating another (p. 90) may apply here. It has also been thought that the increase of connective tissue which takes place in the mammary gland or the prostate under the influence of estrogen may explain the beneficial effect of estrogenic treatment of cancer of these organs (Sirtori and Grattarola, 1947; Lancet, 1947).

As to hormonal treatment in humans we may refer for greater details to the papers quoted and to various chapters of "Endocrinology of Neoplastic Diseases" (1947) especially to those of Nathanson, Farrow, Moore, Dean et al., and Twombly (see also summary of McCullagh, 1948; Huggins, 1949). The clinical tests made whichever their therapeutical results actually have been, must serve as potent stimuli for experimental research with the hormonal treatment of estrogen-induced atypical or tumoral growth though the latter, as has been explained, often differs in fundamental aspects from the neoplasm of the classical terminology of human pathology.

B. HORMONAL TREATMENT OF ESTROGEN-INDUCED UTERINE AND ABDOMINAL FIBROIDS

Experiments with the treatment of abdominal fibroids have been done with progesterone, the most potent steroid in preventing estrogen-induced fibroids.

¹ For a discussion of the implication these findings have for the problem of cancer in general see p. 107.

Experimental fibroids shrink when estrogen is withdrawn (Lipschutz, Iglesias and Vargas, 1938). Important structural changes may become evident as early as three weeks after withdrawal of the hormone; fibroblasts disappear, and the whole tumor is transformed into a sclerotic or hyalinized tissue with small interspersed nuclei (fig. 81). Now, preventive action of steroids has been explained on the assumption that the receptive cells are desensibilized by the afg. steroid so that fibromatogenic action of estrogen cannot take place any more (ch. 14). If this explanation is true an already existing fibroid must respond to the addition of an afg. steroid with shrinking as when estrogen is withdrawn. Full corroboration

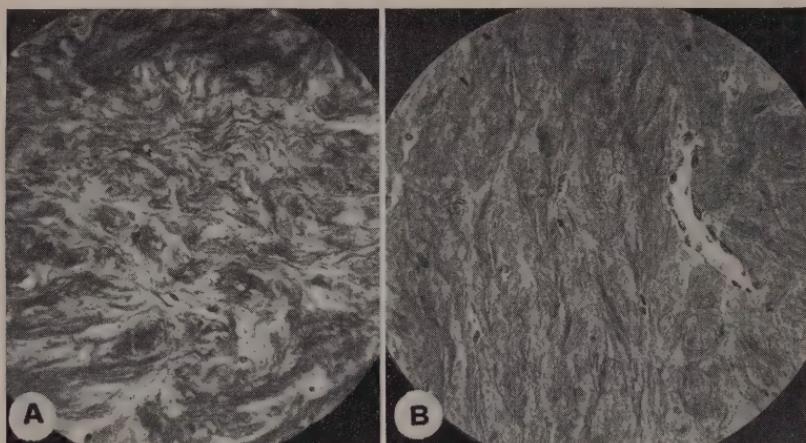


FIG. 81. Fibroids after withdrawal of estrogen. A. Parametrial tumor. Same animal as figs. 61 and 62 (I.14); 113 days after the last injection. $\times 200$. B. Retrorenal tumor. 41 inj. of 80 μg of estrad. (benz.), 110 days; 33 days after the last inj. $\times 200$. (IX.12). In A and B complete absence of spindle shaped cells. Few and scattered small nuclei. Collagenous fibers predominate.

has been given to this (Lipschutz and Maass, 1944; Lipschutz and Schwarz, 1944; Maass, 1942).

Female guinea pigs were implanted with pellets of estradiol; 80 days later pellets of progesterone were added. Necropsy was made 36 to 51 days after that. The average tumoral effect F.T.E. was of 4 with the estrogen acting alone for 80 days, and of only 2 after additional 36 to 51 days of progesterone treatment. When the estrogen has been active for 110 to 130 days without treatment, the average F.T.E. was more than 6. This means that adding progesterone not only prevented the growth of fibroids from 4 to 6 units but caused regression from 4 to 2 (fig. 82). The quantity of progesterone absorbed was of 200 to 300 μg per day.

There was in this work considerable individual variation. But evidence of regression under the influence of progesterone was very strong as seen

from the following details. The average F.T.E. = 4 was reached or surpassed at 80 days of estrogen alone in 13 out of 27 animals. On the contrary, only 5 out of 30 animals reached this average at 110 to 130 days

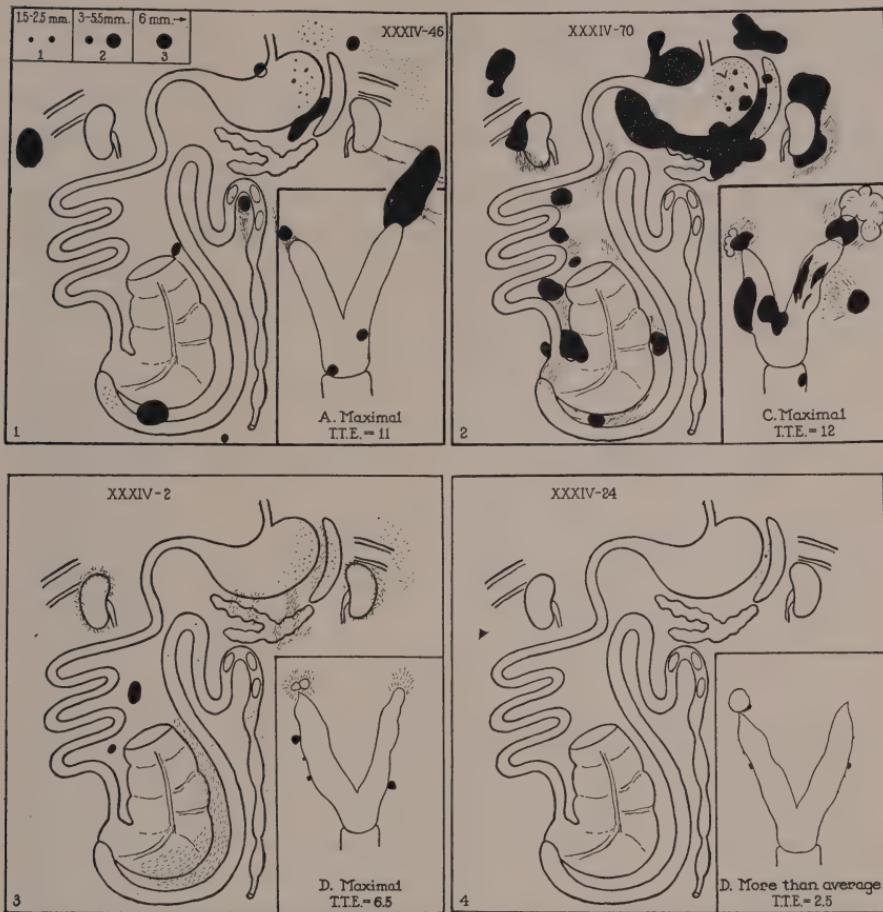


FIG. 82. Treatment of estrogen-induced fibroids with progesterone. 1. Estrad. alone, 69 μ g per day absorbed from subcut. pellet, 84 days. Maximal reaction in the group. 2. Estrad. alone, 34 μ g per day, 130 days. Maximal reaction in the group. 3. Estrad., 37 μ g per day, 115 days; the last 37 days with additional 292 μ g of progesterone per day. Maximal reaction in the group. Compare to 1 and 2. 4. Estrad., 32 μ g per day, 111 days; the last 35 days with additional 309 μ g of progesterone per day. More than average reaction in the group.

with the additional action of progesterone during 36 to 52 days. The tumors still present in some of these animals, 36 to 51 days after progesterone having been added, showed striking structural changes fully identical with those after the withdrawal of the estrogen. After all this there

cannot be the slightest doubt about progesterone causing regression of estrogen-induced abdominal fibroids when being added several months after the beginning of estrogenic action.

C. HORMONAL TREATMENT OF ESTROGEN-INDUCED UTRICULAR FIBRO-MYO-EPITHELIOMA

This tumor of the male guinea pig was prevented with progesterone, desoxycorticosterone acetate or testosterone propionate acting simultaneously with the estrogen (Lipschutz, Yanine, et al., 1945; J. Acuña, 1944; Silberman, 1944). Results are summarized in table 16. Out of 60 guinea pigs receiving estrogen during 7 to 11 months, 25 had an utricular fibro-myo-epithelioma visible to the naked eye. When afg. steroids were added soon after implanting estrogen, there was not a single animal with a

TABLE 16

Group	Action of estrogens*	Action of afg. steroids†	No. of animals		With tumors per cent
			Total	With tumors	
I	<i>months</i> 3½ to 6	0	61	7	11.5
II	7 to 11	0	60	25	42.0
III	7 to 10½	Prevention: 6 to 10 Treatment: 3½ to 4½	25	0	0
IV	10 to 10½		37	5	13.5

* α -estradiol, estradiol-dipropionate, stilbestrol, hexestrol.

† Progesterone, desoxycorticosterone acetate, testosterone propion.

tumor seen to the naked eye. On the contrary, when afg. steroids were added after 5 to 6 months of estrogenic action there were, in a group of 37 animals, 5 cases with tumors. The incidence is the same as in group I; this would suggest that the tumors in group IV were already present when the antifibromatogen was added. Since none of these 5 tumors was large, one might tentatively infer that growth had been stopped by the afg. steroid but that there was no regression. No structural changes were induced in the persisting periutricular fibromyoma comparable to those found in peritoneal fibroids after the additional action of afg. steroids; there was great abundance of smooth muscle bundles often seemingly without any sign of degeneration.

This would be a behavior fundamentally different from that of estrogen-induced abdominal fibroids. How this differential behaviour could be explained? The structure of these two types of experimental tumors is different. The tumor of the abdominal serosa is often an almost pure

fibroid and only rarely a true myoma; on the contrary, in the periutricular tumor myomatous tissue predominates. One might tentatively suggest that there are *quantitative* differences of response to afg. steroids between the two types of tissues of which these experimental tumors are built up. Or should one assume that there is an essential difference in the behavior of these two tumoral tissues? Such an assumption has been made by Segaloff et al. (1949) in whose work progesterone did not cause regression of uterine fibromyomata in women. Since these tumors enter into regression when ovarian function ceases in the menopause and apparently shrink under the influence of testosterone clinical trials with *larger* quantities of progesterone shall be of interest. In the work of Segaloff et al. about 5 mg per day was absorbed from subcutaneously implanted pellets, or about 80 μg per 1 kg of body weight. The corresponding figure in our experiments with the treatment of experimental fibroids in guinea pigs was about 400 μg ; and we do not know whether regression would still take place with those small quantities which were effective in preventing these tumors.

D. ON THE BEHAVIOR OF ESTROGEN-INDUCED ATYPICAL EPITHELIAL PROLIFERATION TOWARDS ANTIFIBROMATOGENIC STEROIDS

It is known since long that estrogen-induced atypical epithelial proliferation can be prevented when progesterone or testosterone are acting simultaneously. We may refer to prevention of squamous metaplasia of the uterine cervix in rhesus by an extract with progestational action (Hisaw and Lendrum, 1936); of cystic glandular hyperplasia of the endometrium in rhesus by testosterone (Zuckerman, 1936b); of the metaplastic changes of the utriculus in rhesus by androstanediol and in several cases also by progesterone (Zuckerman and Parkes, 1936a). Other examples are given in table 15. In our guinea pigs adenomatous growth of the endometrium also has been prevented with different steroids (see p. 153). Whereas we have made no special study with the *treatment* of the atypical growth of uterine epithelium, we have studied the behavior of epithelial structures of the utricular fibro-myo-epithelioma persisting after about 4 months of action of afg. steroids (group IV in table 16). These epithelial structures were in all respects similar to those found in animals in which the estrogen acted alone. There was squamous stratified epithelium with cornification, with concentric disposition of cells and formation of pearls. There was papillomatous growth filling the cystically enlarged utriculus or vas deferens. But here again, as with the myomatous part of the utricular tumor, one must emphasize that persistence of an estrogen-induced atypical epithelial structure in spite of progesterone or desoxycorticosterone treatment might have been due to the *quantities* of these anti-fibromatogens having been insufficient.

Chapter 16.

ANTITUMORIGENIC ACTIONS OF STEROIDS, AND THE CONCEPT OF THE MOSAIC OF TERRITORIES

Stress has been laid in chapter 7 of the First Part on the experimental statement that each tissue of an animal follows as to tumoral responses its own law. This applies to the homologous cells of the same tissue, or to the homologous tissues of different species. The organism behaves, as to its reactivity to tumorigenic stimuli, like a mosaic of territories. This concept links reactivity to pathological tumorigenic stimuli with reactivity to physiological morphogenic stimuli. It is of fundamental interest that the same concept applies also to antitumorigenic stimuli. We have reported that in guinea pigs with the simultaneous action of estrogen and afg. steroids the nipples and the mammary glands developed equally if not more than with estrogen alone (p. 153); similar statements have been made by Von Wattenwyl (1944). Metaplasia of alveolar cells of the mammary gland which occurs in the guinea pig under the prolonged influence of estrogens was in our work seemingly more frequent with simultaneous action of testosterone and estradiol than with estradiol alone (fig. 83). This result might be due to estrogenic action of large quantities of testosterone (Deanesly and Parkes, 1936); even metaplasia of the endometrium can be produced when sufficient quantities of testosterone are given (Selye and Clarke, 1944).

Contrary to our clear cut results with benign mammary growth in *guinea pigs* are those with mammary adenocarcinoma in *mice* where growth can be arrested with testosterone (Lacassagne and Raynaud, 1939; Nathanson and Andervont, 1939; Loeser, 1941; et al.; table 15). But there are great discrepancies in the behavior of different strains in mice. Progesterone was active in preventing to a certain degree mammary cancer in the R3 strain (Heiman, 1945) by reducing the incidence from 54 to 17 per cent; but the same steroid had no influence on mammary tumor formation in the Marsh-Buffalo strain (Bischoff and Rupp, 1946) or the C3H strain (Burrows and Hoch-Ligeti, 1946). There are even reports about progesterone enhancing the incidence of mammary cancer

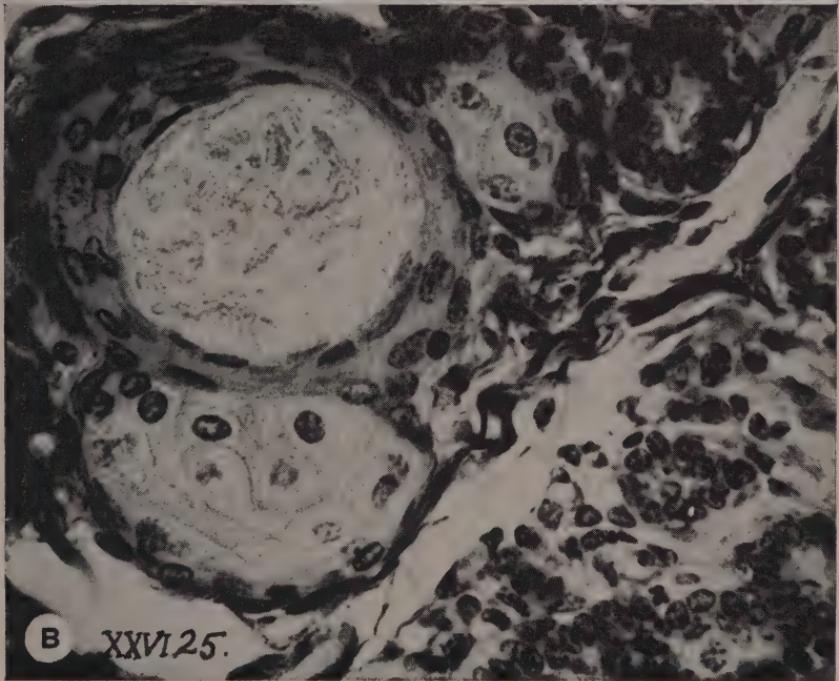
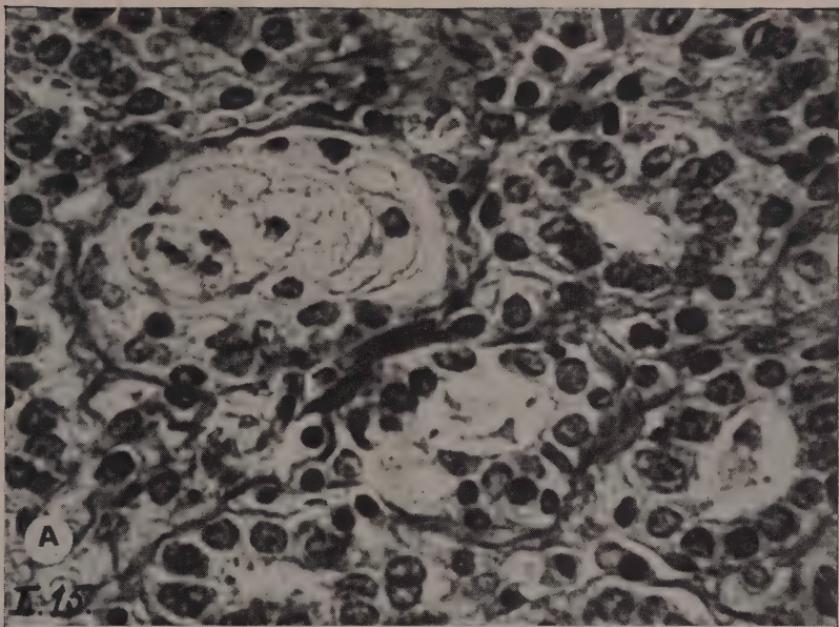


FIG. 83. Metaplastic acini in mammary fibroadenoma. A. Same animal as fig. 50. $\times 800$. B. Simultaneous action of estrogen and testosterone propion. 36 μ g of estrad. and 202 μ g of testerol. per day, subcut. pellets, 55 days. $\times 500$. (XXVI.25).

in mice (Symeonidis, 1948) and rats (Cantarow et al., 1948). In the guinea pig testosterone is less afg. and also less antiestrogenic than progesterone (table 14); but in *rats* and *mice* testosterone is more active in inhibiting proliferation of the vaginal epithelium than progesterone (Courrier and Solal, 1937a, 1937b; Robson, 1938a). The antagonizing action of various steroids on the estrogen-induced hypophysial increase¹ has already been referred to (pp. 153 to 155). Testosterone but also the non-androgenic cis-testosterone are the most powerful antagonizers; the action of progesterone is doubtful as to antagonizing both the initial increase (Albert and Selye, 1942; but see also the results of Smith and Smith, 1944) and the tumoral hypophysial growth (our work, see p. 153).

All these results show that, as with the tumorigenic action of the steroids, each territory follows its own law also with the afg. action of these compounds.

Importance must be attached to these comparative statements when trying to apply experimental results with the afg. actions of steroid hormones to treatment in human beings. The findings with the mammary gland may serve as an example: good results with testosterone have been claimed by different authorities in mammary cancer in women—a behavior similar to that with mammary cancer in mice and contrary to that with estrogen-induced mammary proliferation in guinea pigs. We must not be too pessimistic but neither too optimistic as to the clinical application of experimental statements on the antitumorigenic action of steroids!

PART III.

The Steroid Balance,
or Steroid Homeostasis,
and the Antitumoral Autodefense

INTRODUCTION

We have examined in Part I the quantitative and timing conditions under which estrogens, physiological morphogenic substances the female organism needs for the upkeep of its functions, can acquire tumorigenic and even carcinogenic faculties. In Part II the antitumorigenic faculties of certain steroid compounds as progesterone, cortical steroids and others have been studied. The question arises about the means the female organism may dispose of to protect itself against the estrogen becoming toxic and tumorigenic, and whether the organism makes use of the anti-tumorigenic faculties of steroids for this very physiological purpose. On the basis of the now available knowledge on the fibromatogenic and afg. actions of steroids the tentative assumption has been made that the body, by adjusting its steroid balance, disposes of a complex system of an antitumoral autodefense (Lipschutz, 1943a). Though based on experimental facts the concept of a steroid homeostasis for antitumoral autodefense is certainly *hypothetical*. The concept is solely to serve as a guide in further research, both experimental and clinical. Since first exposed it has been supported by new relevant experimental statements.

One must assume from the onset that many organs and still more steroids partake in the steroid homeostasis for antitumoral autodefense. We shall deal separately with three different mechanisms: the hypophysial-ovarian factors, the suprarenal and hepatic factors.

Chapter 17.

HYPOPHYSIAL-OVARIAN FACTORS OF THE STEROID BALANCE

A. RHYTHMIC PRODUCTION OF ESTROGENS

The concept of hypophysial-ovarian factors at the service of antitumor autodefense is based on three well established facts: *first*, discontinuous production of estrogens, or rhythmic alternation of the quantity of estrogen produced, is in all mammals the normal condition; *second*, this rhythmic alternation is dependent upon an hypophysial-ovarian interrelationship; *third*, the estrogen acquires tumorigenic faculty when allowed, contrary to nature, to act continuously. On the basis of the first and third of the above statements—one physiological and the other experimental—one may reasonably consider the sexual rhythm in the mammal as fundamental for the autodefense of the body against the tumorigenic actions of the endogenous estrogen.

Atypical epithelial proliferation, hyperplasia and metaplasia, and invasion of the myometrium by proliferated glands, was elicited with minute quantities of estradiol when administered in such a way that there was continuous action as with the injection of an ester of slow absorption, (p. 38), or with the subcutaneous implantation of a pellet containing but a small percentage of the free hormone (p. 41 to 44, 116 to 117). An even more striking example was offered by experiments in which large quantities of esterified estrogen were no more able to elicit these tumors when administered under timing conditions which did not allow for continuous action (p. 41 and fig. 33). A glance at a graphic representation of these experiments is convincing: the sexual rhythm in mammals, besides its significance in reproduction, may serve as an autodfensive device in the body.

The interest this statement, derived from experimental observation, arouses in the gynecologist can not be overestimated. It furnishes an impressive example, or model, of what the normal sexual rhythm may mean in the physiology and pathology of the woman, besides the part it has in reproduction.

B. RHYTHMIC PRODUCTION OF PROGESTERONE

Rhythmic variation in the production of estrogen is but one-half of the ovarian endocrine function in the mammal; production of progesterone also is rhythmic. Production of progesterone may tentatively be interpreted as another means of a bodily antitumoral autodefense since progesterone has been recognized as the most potent afg. steroid. Is intermittency of the secretion of progesterone not contrary to our interpretation? Will progesterone when allowed to act only intermittently, or discontinuously, still prevent abdominal fibroids due to the continuous action of estrogen? That this is the case has been shown by the following experiment (Iglesias, Lipschutz and Nieto, 1944). Pellets of estradiol dipro-

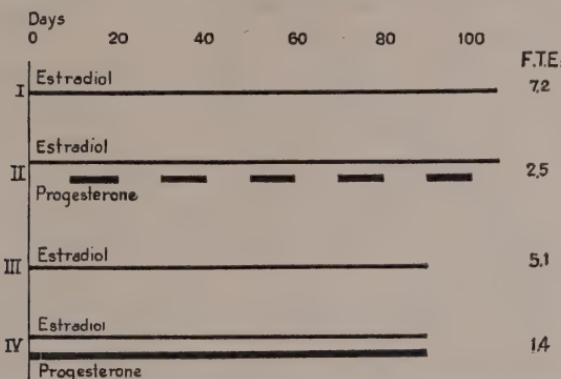


FIG. 84. Discontinuous and continuous action of progesterone against estradiol. Preventive action in both groups. Prevention was less complete in the discont. group, though quantities of progesterone twice or thrice greater were absorbed; possibly due to the longer duration of the experiment in the discont. group.

pionate were implanted subcutaneously into female guinea pigs into which pellets of progesterone were implanted ten days later. Whereas estradiol was allowed to act continuously the pellets of progesterone were again withdrawn 10 days later, to be again implanted after 10 days, and so forth. By this alternating action of progesterone fibroids were prevented or considerably diminished (fig. 84).

The afg. action of progesterone here referred may be due to a desensibilization of the tissues reacting to the estrogen though this is so far only a tentative suggestion (p. 157). But there is evidence that this is not the only mechanism by which the antagonistic interplay of the two steroids comes into being.

C. THE ESTROGEN-PROGESTERONE ANTAGONISM

It has been assumed since long that the gonadotrophic function of the anterior lobe is controlled by the sex hormones. This problem is of funda-

mental interest from the point of view of the steroid homeostasis in the body and of antitumoral autodfense.

Differential actions of *estrogens* and androgens, especially testosterone, on the cytology of the anterior lobe have been amply studied (Wolfe et al., 1937-1941; Martins, 1936a, chap. X; Sevringshaus, 1939; summary Lipschutz, 1942d; Selye, 1947b, p. 257). The influence estrogen has on the gonadotrophic activity of the anterior lobe is now well known (references in Merckel and Nelson, 1940; Courrier, 1945; Cole, 1946; Masson and Barsantini, 1948; Robertson, 1948; Selye, 1947b, p. 383; Burrows, 1949). One of the most striking actions of large doses of estrogen is the stimulation of luteinization as discovered first in mice (Hohlweg, 1934; Hohlweg and Chamorro, 1937). It was but logical to attribute this result to an enhanced delivery of the luteinizing hypophyseal hormone (LH). But estrogen maintains the corpora lutea in the hypophysectomized pseudopregnant rabbit (Westman and Jacobsohn, 1937; Robson, 1937; quoted from Robson, 1938b);* pregnancy continues in absence of the hypophysis when estrogen is given (Robson 1938b). Contrary to these results in the rabbit are those in hypophysectomized rats (Nelson and Pichette, 1942).

Changes in hypophysial cytology induced by *progesterone* have apparently not been described. But various authorities have reported that progesterone inhibits luteinization, or the release of hypophysial LH, in mice, rats and guinea pigs (Selye, Brown and Collip, 1936; Dempsey et al., 1936; Dempsey, 1937; Astwood and Fevold, 1939; Selye, 1939).** No inhibition of luteinization took place when progesterone was given to pregnant mice (Selye, 1939) the placenta and not the hypophysis being the source of the luteinizing hormone in pregnancy.

Enhancement of the metabolic conversion of estradiol by progesterone also has been reported (Smith and Smith, 1946).

New knowledge of an antagonistic interplay of estrogen and progesterone in the control of follicular development has been recently obtained with a simple device—the intrasplenic autoplasic graft of the ovary with the removal of the second, and administration of steroids. Our work with intrasplenic ovarian grafts (fig. 85) was but an extension of the funda-

* Estrogen has no such action in the hypophysectomized rat; but when prolactin is given simultaneously with estrogen the synergic action of the latter as to luteinization becomes evident (Desclin, 1949b).

** On the contrary, various workers found increased luteinization when progesterone was administered to rats. But the disparity may be due to those special ovarian conditions which prevailed in these experiments: a strain of rats with persistent estrous without spontaneous corpus luteum formation (Everett, 1940), or male rats with ovarian grafts (Kempf, 1949) luteinization being absent or almost absent in the graft (see also summary of Hisaw, 1947).

mental discovery of the Biskind group that estrogen absorbed from a pellet implanted into the spleen is inactivated on account of its being drained through the liver (Biskind and Mark, 1939). There was also the former statement that ovaries when grafted into the portal field do not manifest any endocrine activity (Golden and Sevringshaus, 1938).

When one ovary is removed from the guinea pig and the other one is grafted into the spleen of the same animal, the graft takes in almost every case, as has been shown in several hundreds of experiments in our Department since 1942 (Ponce de León, 1944; Woywood, 1944; Gay, 1944;

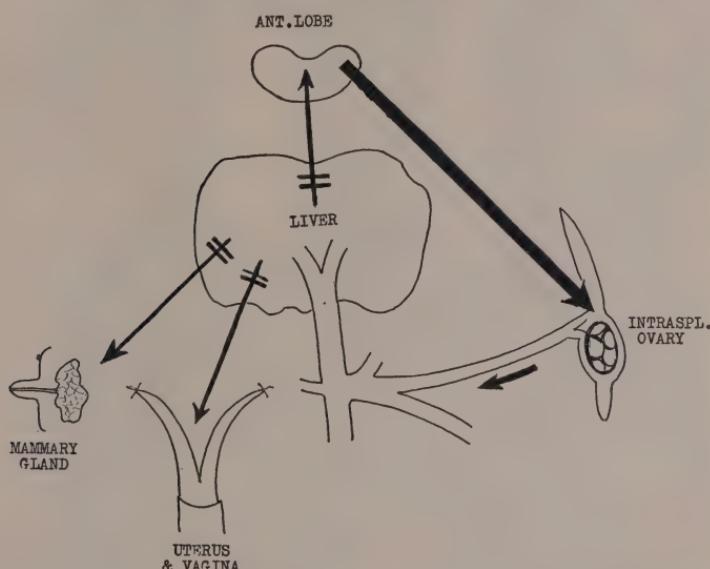


FIG. 85. Functional relations between ovary and hypophysis in experiments with intrasplenic grafts. The graft is reached by gonadotrophic hormones, but estrogen produced is destroyed in the liver. The hypophysis freed from the ovarian control stimulates follicular development and production of hemorrhagic follicles.

Meza, 1945). The graft may survive for almost two years.¹ At two months after transplantation the spleen becomes distended by the graft (fig. 86A); the graft contains blood follicles (fig. 87B) similar to those which can be produced in certain species by the administration of chorionic or hypophysial gonadotrophins.² The condition is due to the hypophysis

¹ Taking and survival is considerably less with intrasplenic *homoio-transplantation* (Lake, 1945; Contreras, 1946).

² Blood follicles in ovarian grafts of the portal field have been recently reported also in mice (Vivien, 1948b; intrasplenic grafts) and in rabbits (Mayer and Soumireu, 1948; mesenteric grafts). So far as I am aware the rabbit is the only animal



FIG. 86. Distension of spleen by ovarian graft in castrated female g. pig. A. 63 days after grafting. (LII.56). B. 97 days. (LII.64). A and B, size 1.2. C. 303 days. $\times 2$. (LII.77).



FIG. 87. Autoplastic ovarian grafts in the spleen of castrated g. pigs, and grafts in animals with one ovary in situ. A. One ovary in situ. No distension of the spleen by the graft. A luteinized follicle, or corpus luteum, with a small blood point; the only one with some bleeding in a series of 21 animals. 60 days. (LXXI.109). B. Castrated, 62 days. Distension of the spleen. Four hemorrhagic follicles. (LXXI.94). C. Castrated, 303 days. Hemorrhagic follicle and many corpora lutea filling the whole ovary. (LII.75). Size of A, B and C, $\times 3$.

being freed from the ovarian control. Blood follicles fail to appear in intrasplenic grafts when the second ovary has been left at its normal place

in which blood follicles can be seen under normal conditions (Hammond and Marshall, 1925). They are elicited in the virginal animal when chorionic gonadotrophins or hypophysial hormones are administered (the Friedman test of pregnancy); it is the same with the infantile mouse (the Aschheim-Zondek test). No mention is made of blood follicles in the guinea pig in which extensive work has been done both with chorionic and hypophysial gonadotrophins by Aron, Loeb, Guyénot and Ponse (see Hamburger and Pedersen-Bjergaard, 1946; full references). But blood follicles may occur exceptionally also in the guinea pig when gonadotrophins are administered (Bärtschi and Ponse, 1934, fig. 6 on plate III). We have seen blood follicles also in intrarenal ovarian grafts though only occasionally (Lipschutz, 1928, 1929; Lipschutz, Martins and Viñals, 1933).

(fig. 87A). The graft may then offer at 2 months the aspect of a normal ovary with Graafian follicles and corpora lutea which were found in no less than half of these ovaries (Gay, 1944; Lipschutz, Ponce de León et al., 1946), even at 15 months (Niedmann, 1947; Bruzzone, Lipschutz and Niedmann) (fig. 88B). Taking and survival diminishes indeed in non-castrated females.

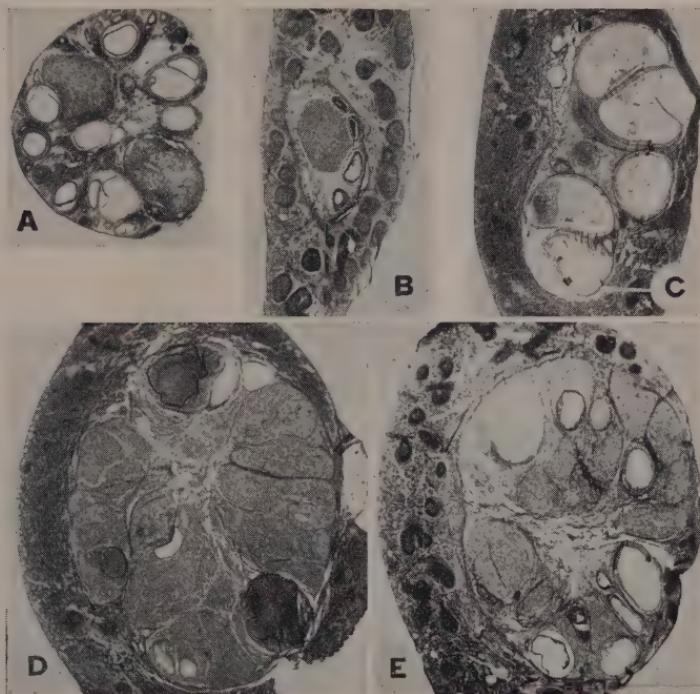


FIG. 88. Intrasplenic ovarian grafts at different times after grafting. A. Normal ovary, one of the largest of our collection. Two large corp. lut., many Graaf. foll. (Ch.278). B. One ovary in the spleen, the other *in situ*. Corp. lut. and various Graaf. foll. (LXXI.105). C. Castrated, 62 days. Various cystic foll.; cystic hemorrh. foll. (second from beneath). (LII.65). D. Castrated, 303 days. Various corp. lut.; two cystic hemorrh. foll. (LII.78). E. Castrated, 654 days. Various corp. lut.; there were also hemorrh. foll. but not visible in this section. (LII.73). All figures $\times 5$.

The behavior of the graft with the second ovary *in situ* has been described also in the rat (G. R. Biskind and Biskind, 1948); it has been compared to that of an ovarian graft in an hypophysectomized animal (G. R. Biskind, Pencharz and Biskind, 1947). This is not the case in the guinea pig; though here also the graft was seemingly in a position less advantageous than that of its companion *in situ*.

The condition of the intrasplenic ovarian graft in a castrated guinea pig changes fundamentally when ovarian steroids are administered

(Húmerez, 1946; Lipschutz, Iglesias, Bruzzone, Húmerez and Peñaranda, 1948b). With estrogen absorbed from a subcutaneously implanted pellet production of blood follicles was inhibited (fig. 89B). On the contrary, luteinization was greatly enhanced—there was no animal without corpora lutea; their number and size increased, and the volume of luteal tissue was several times that without estrogen. Progesterone does not inhibit

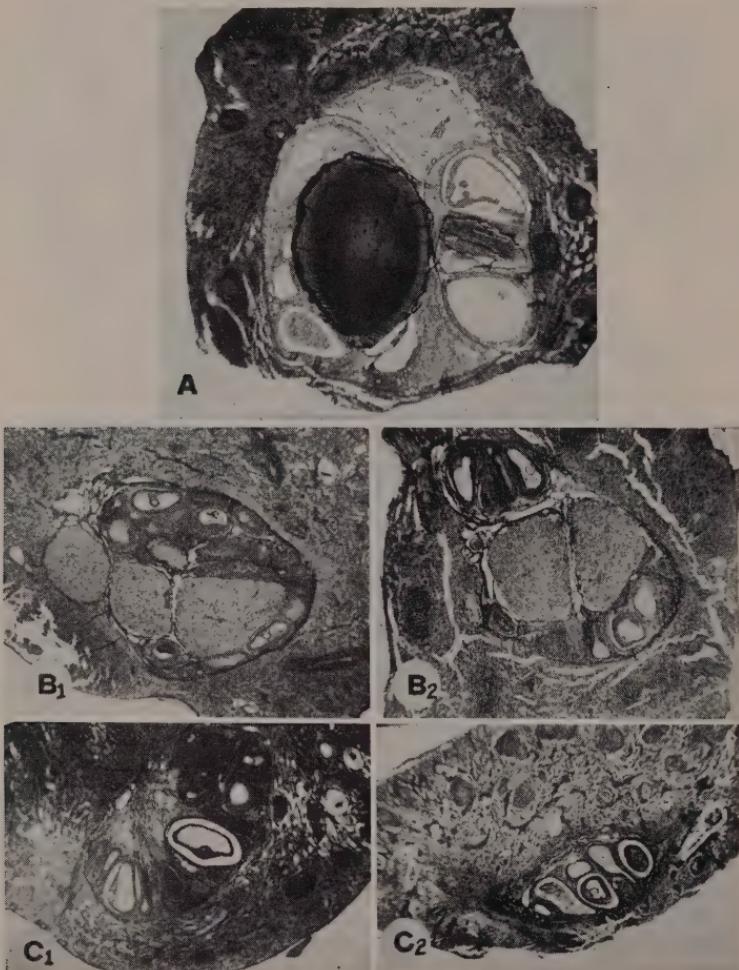


FIG. 89. Control of ovarian-hypophyseal relation by steroids. Intrasplenic grafts, 60 to 65 days after grafting, 39 to 49 days of action of steroids absorbed from subcut. pellet. A. Progesterone: hemorrhagic foll. present in 14 out of 23 animals; corp. lut. present but in 2. Distension of spleen. (CI.85). B₁ and B₂. Estradiol: hemorrh. foll. absent in all the 12 animals of the series; corp. lut. present in all animals; foll. small. No distension of spleen. (CI.26 and 17). C₁ and C₂. Estradiol and progesterone: hemorrh. foll. absent in all the 14 animals of the series; corp. lut. present but in 1 animal; follicles small. (CI.94 and 97). No distension of spleen. All figures $\times 10$.

blood follicles (fig. 89A). A new and quite unexpected ovarian pattern was established when progesterone was given simultaneously with estrogen (fig. 89C). There were no blood follicles, that is to say, estrogen continued to control follicular development; but the luteinizing action of estrogen was almost completely counteracted by progesterone (fig. 89C).

Progesterone acts as an "antiluteinizer"; desoxycorticosterone acts similarly to progesterone (Peñaranda, 1947; Lipschutz, Iglesias et al., 1948b).³ This antiluteinizing faculty is patent not only when luteinization is induced by the administration of estrogen; progesterone counteracts also excessive luteinization which establishes itself spontaneously in intrasplenic grafts many months old (Iglesias, Lipschutz and Mardones, 1948, 1950a).

There is thus full evidence that, *under the given experimental conditions, estrogen calls for luteinization and progesterone counteracts it.* One may assume that both these steroids interfere in the release of the prehypophysial gonadotrophic hormones, and that there is an *intrahypophysial* antagonistic play of these steroids.

On the other hand, one may claim that the interplay of the two steroids takes place in the ovary itself; luteinization once started can be maintained with estrogens in the absence of the hypophysis (see p. 176). But in the hypophysectomized rat the ovarian changes induced with estrogen are just the opposite of those in intrasplenic grafts: follicular development was enhanced by estrogen in the hypophysectomized rat (Pencharz, 1940; Williams, 1940, 1944, 1945; Simpson et al., 1941; Gaarenstroom, 1942; Gaarenstroom and De Jongh, 1943; Desclin, 1949a), and guinea pig (M. Aron et al., 1948), and so also in the infantile intact mouse (Bullough, 1942).⁴

Whatever the role of the direct action of estrogen on the ovary may be, it would not be at variance with the concept of an *intrahypophysial* antagonistic play of the two essential ovarian steroids. Maintenance of a high estrogen level in the body is certainly counteracted by the estrogen itself the latter promoting luteinization, and protection against continuous—toxic and tumorigenic—action of the estrogen is thus procured. The interplay between estrogen and progesterone in the ovarian-hypophysial functional complex might be considered as a fundamental autodifensive antitumoral device.

³ In older work of Selye and Friedman (1940) in the rat with the simultaneous action of estradiol and progesterone, or desoxycorticosterone, corpora lutea persisted. This may have been due to special quantitative conditions.

⁴ In more recent work it has been shown that, in the guinea pig, the effects of the direct action of estrogen on the intrasplenic graft (pellets of estrogen in the ovarian graft, or near it in the spleen) are fundamentally different from those due to subcutaneously implanted pellets of estrogen (Tglesiás, Lipschutz and Rojas, 1950; Rojas, 1950).

The very relevant question arises whether the organism is liable of disposing, in the follicular and luteal ovarian phases, of the luteinizing threshold quantity of estradiol and the antiluteinizing threshold quantity of progesterone. The luteinizing action of estrogen is most probably due to those relatively large quantities of the hormone which are secreted at a given moment of the cycle and not to those minute quantities which are released continuously (see discussion in Barahona et al., 1950, based on our work and that of Furlong et al., 1949, in guinea pigs; for the rat see Jungck et al., 1947; Heller and Jungck, 1947). The antiluteinizing faculty of progesterone was manifest with about 40 to 50 μg per day though there was full evidence of antiluteinizing action also with doses much smaller than that (Mardones, 1948; Iglesias, Lipschutz and Mardones, 1948). But we do not know whether the physiological level has been exceeded, or not, with this quantity.

The steroid control of the gonadotrophic function of the hypophysis seems to be a definitely established fact notwithstanding the doubts still existent as to the quantitative aspects of this control, and as to other mechanisms possibly involved.* The importance of this control cannot be overestimated. But the current concept that the gonadotrophic activity of the anterior lobe is "inhibited" by estrogen must be dropped; it was an oversimplification. However, the steroid control is certainly not the only mechanism by which the gonadotrophic activity of the hypophysis is regulated by the ovary. In experiments with the partial removal of the ovary—years ago, before hypophysial gonadotrophins became known—the conclusion was reached that follicular development is quantitatively regulated by a balance between production of extraovarian X-substances and their use in the ovary; the number of follicles which ripen, or of the ova shed, does not diminish, or diminishes only slightly, when one, or more than one, ovary is removed (Asdell, 1924; Hartman, 1925; Hammond and Marshall, 1925; Lipschutz, 1925, 1927b, 1928). These ideas were but a further development of those of Heape (1906) and of Sand (1919).⁵ The conclusion was also reached that "cystic degeneration of the ovary in all the different forms observed in human and animal pathology is possibly never of purely ovarian origin but rather caused by an upset in the balance of production and use of X-substances" (Lipschutz, 1928). More recently

* For the neural factor the reader may be referred to the work of Sawyer et al. (1949) and Kehl and Molina (1949).

⁵ John Hunter (1787) was the first to give experimental evidence in the sow that after removal of one ovary the size of the litter remains almost the same as in the normal animal. He concludes prophetically that this is due to the "constitution," or to hypophysial gonadotrophins in modern terms.

this mechanism of control has been given attention in work with intra-splenic ovarian grafts in the rat. Jungek et al. have discovered that the hypophysis of the rat with these grafts, when administered to infantile females, did not elicit so considerable an ovarian increase as does the hypophysis of the castrated rat; the result has been explained on the assumption that production (or release?) of gonadotrophins is regulated through their consumption in the ovary (Jungek et al., 1947). We shall discuss again this relevant question in chapter 21, B, 1.

Chapter 18.

EXPERIMENTAL OVERTHROW OF THE OVARIAN-HYPOPHYSIAL RELATIONSHIP

We have referred in the First Part to atypical proliferation of the uterine glands in the guinea pig induced by an operative interference on the ovary, the so called "*ovarian fragmentation*" (p. 4, 88). The purpose of these experiments which were started almost 30 years ago (Lipschutz and Voss, 1925), was originally to study the compensatory ovarian reaction after the removal of one entire ovary and of the greater part of the second ovary. But these experiments turned out soon and unexpectedly to be a fascinating chapter of experimental pathology of the ovary (Lipschutz and Osnovikoff, 1932; Lipschutz, 1936a, 1937b, 1938). In similar animals remarkable mammary, vaginal and uterine changes may take place. Results are unsteady; the changes produced vary greatly from one series to another, or in the same series; they may fail to appear at all. Sometimes they appear at a late date. The most significant results were obtained with females operated when fully grown or at an advanced age and necropsied almost three years after the operative interference.

Let us take first the changes in the *nipples* and *mammary glands*. The nipples may grow in the course of several weeks; in some cases they are more developed than during gestation. There was also pigmentation around the nipples. Mammary glands increased greatly and, though indeed only very exceptionally, tiny drops of colostrum could be obtained by pressure on the basis of the nipples. The condition of the nipples and mammary glands here described, including pigmentation, are known to be due in the guinea pig to the prolonged action of estrogen. The behavior of the *vaginal wall* was in agreement with the assumption that prolonged action of estrogens was here in play. Whereas cornified cells appear during estrus in the vaginal smear of the normal guinea pig only for several hours, as known since the classical work of Stockard and Papanicolaou, they may be found in females with ovarian fragmentation for 10 days or more. The vaginal opening may enlarge and a viscous secretion, sometimes of a reddish color, may be stated. Exceptionally there may be even manifest genital bleeding. After a certain time the cornified cells are replaced in the vaginal smear by great vacuolated cells typical of the second half of

pregnancy. At microscopical examination a thick vaginal wall may be found, with very pronounced epidermization, whereas in other cases the vaginal mucosa is transitional between estrus and pregnancy. In many cases, especially when the operation has been performed at an early age,

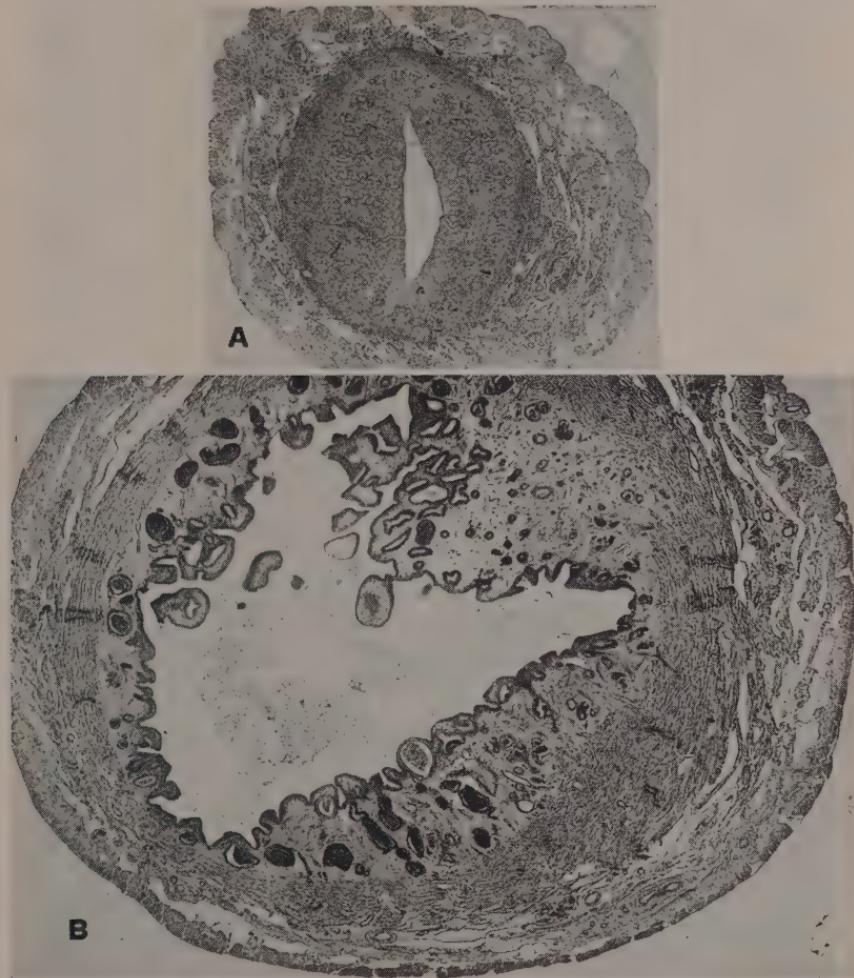


FIG. 90. Uterine mucosa after ovarian fragmentation in g. pigs. A. Normal uterus, for comparison with B. $\times 15$. B. 32 months after fragmentation. Small glandular cysts with pus. Digitations and polyps filling the cavity. Hypertrophic myometrium. $\times 15$. (Ch.1251).

nothing abnormal happens seemingly in the vaginal region. It may remain closed for a long time to open more or less regularly. But after about a year things may change the vaginal condition as described above becoming manifest.

When the experiment is prolonged for about three years, sometimes sooner, the *uterus* may be found greatly increased; its weight may reach the

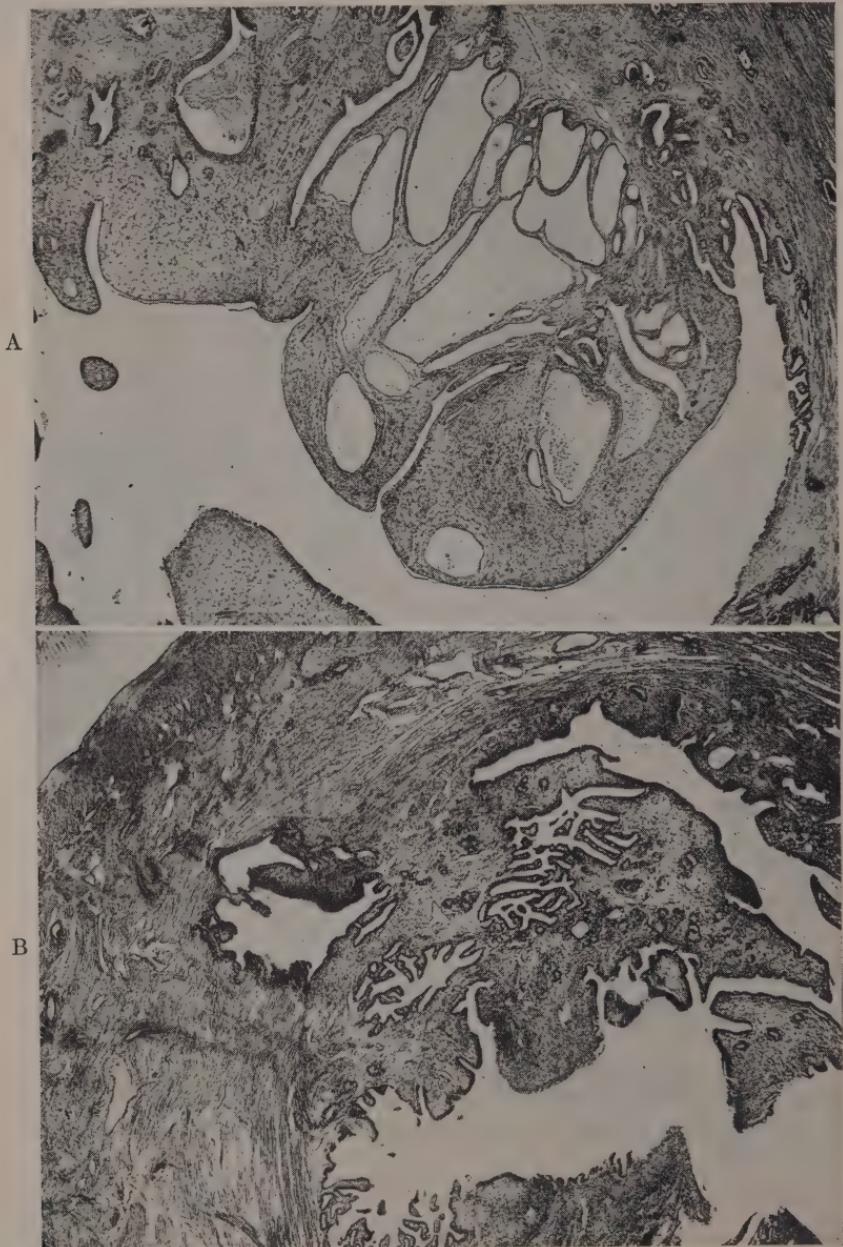


FIG. 91. Uterine polyps after ovarian fragmentation (Ch.1253). A. Polyps and cysts. $\times 15$. B. Digitations in the cysts. Cystic gland pressing against the myometrium and displacing it. $\times 15$.

triple or even the twentyfold, or more, of the normal uterine weight. Pyometra may occur. Significant changes were found in the uterine

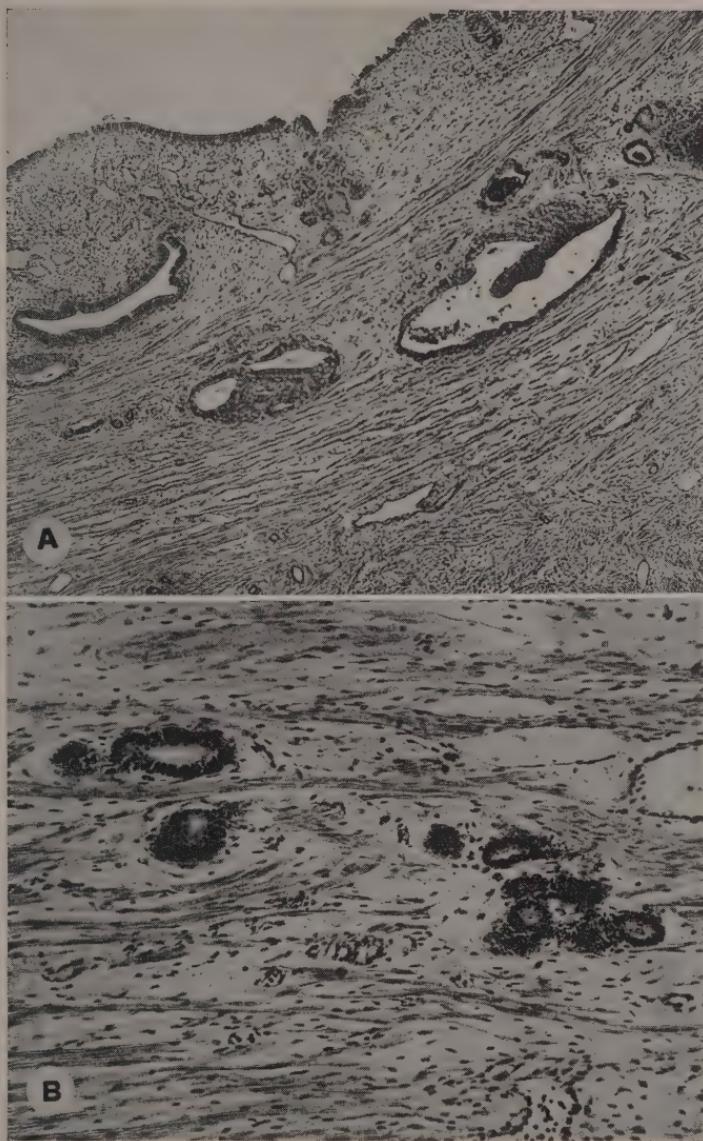


FIG. 92

mucosa, as cystic glandular hyperplasia (fig. 90) and large polyps (fig. 91). Digitations may be found in distended glands (fig. 91B and 92D). The proliferated glands infiltrate the myometrium (fig. 92 and 93). There

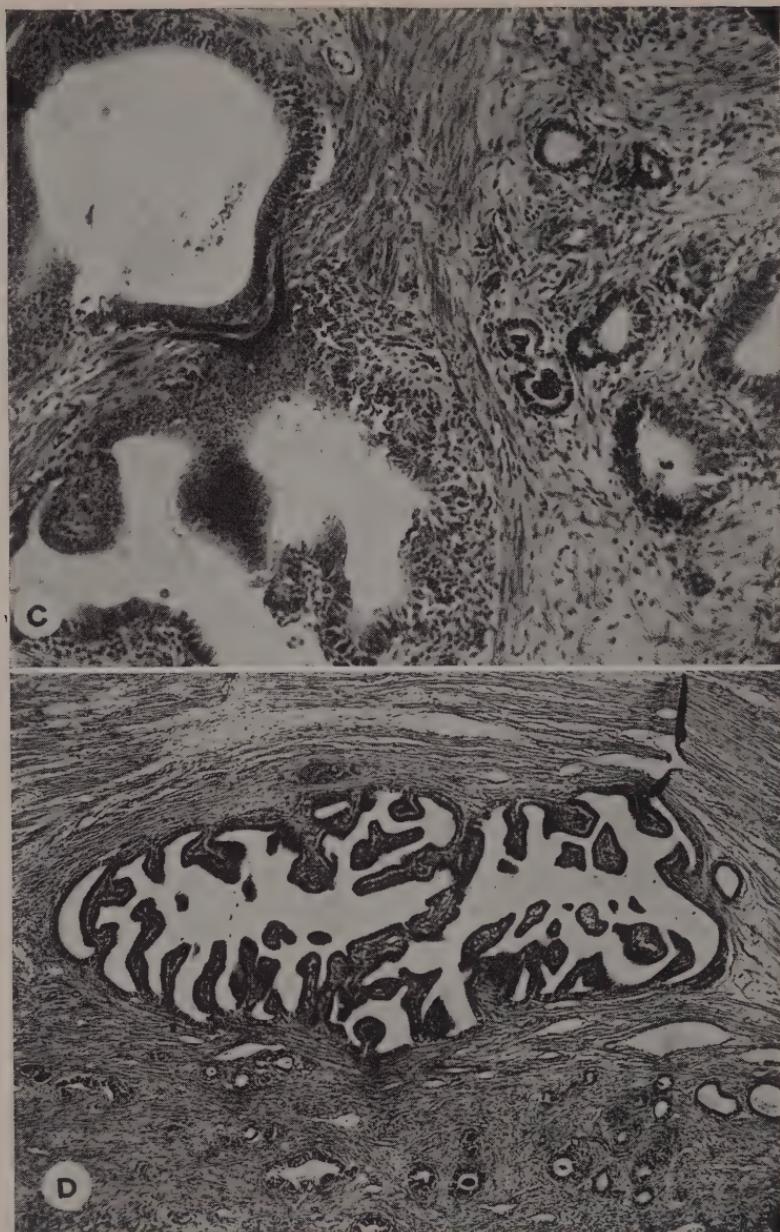


FIG. 92. Invasion of uterine glands into the myometrium after ovarian fragmentation. Same animal as fig. 91. A. Glandular cysts in the circular layer. The longitudinal layer is seen in the right corner. $\times 38$. B. Non-cystic glands in an edematous part of the circular layer. $\times 90$. C. Glands of different conditions in the myometrium. $\times 90$. D. Large glandular cyst in the circular layer. Epithelial digitations. $\times 38$.

were metaplastic changes in the uterine horns and the *cervix*; the individual cervices may become cornified. In one case there was in the cervix a nodular epitheliomatous proliferation (figs. 94 and 95).



FIG. 93. Invasion of uterine glands into the myometrium after ovarian fragmentation. A. 31 months after operation. Cluster of glands between the circular and longitudinal layers. Condition comparable to adenomyosis. $\times 70$. (Ch.1295). B. 33 months after operation. Gland traversing the circular layer. $\times 70$. (Ch.1256). In both these cases the increase of the uterine weight was not very large: only 2.1 and 1.8 g respectively.

All this suggests that the normal sequence of the two ovarian phases has been disturbed. In most of the cases the *ovarian remnant* is similar to a normal ovary. It contains fully grown follicles and corpora lutea; the

number of the latter may reach as much as 5 which is more than the average number in two intact ovaries, in compliance with the law of follicular constancy. But in other cases luteic cysts have been found as in the cat (fig. 104), the guinea pig (fig. 105) and the rat, though indeed only exceptionally. The luteal cyst contained in one case blood (fig. 106). It seems reasonable to assume that these ovarian changes were due to an overthrow of the normal ovarian-hypophysial relationship. Other experimental statements also were in favor of this assumption. The anterior lobe of these abnormal guinea pigs, when administrated to infantile rats



FIG. 94. Epitheliomatous proliferation of the mucosa of the individual uterine cervix after ovarian fragmentation. Same animal as figs. 91 and 92 (Ch.1253). Partial epidermization with cornification. Atypical proliferation with formation of solid cords and pearls. $\times 20$.

and mice, was seemingly more active than that of normal females (Lipschutz, 1937b, 1938). Similar statements are indeed not very reliable: there are great quantitative variations with the gonadotrophic activity of the anterior lobe, especially in guinea pigs.

We may conclude from the above that highly pronounced atypical proliferation of the uterine mucosa, including invasion of the proliferated glands into the myometrium and disorderly epitheliomatous proliferation of the cervical mucosa, can be elicited in the guinea pig when the normal sequence of production of estrogen and progesterone, or the balance be-

tween these steroids, is disturbed by an experimental overthrow of the ovarian-hypophysial relationship. One may tentatively suggest that a similar mechanism of neoplastic growth is operative in the human body. The pioneer work of Hofbauer (1930, 1931; see 1939) with the production of cystic glandular hyperplasia in the guinea pig through the administration of anterior lobe may be mentioned here.

Similar statements on ovarian fragmentation and its sequels in the genital tract of the guinea pig have been made by various workers as

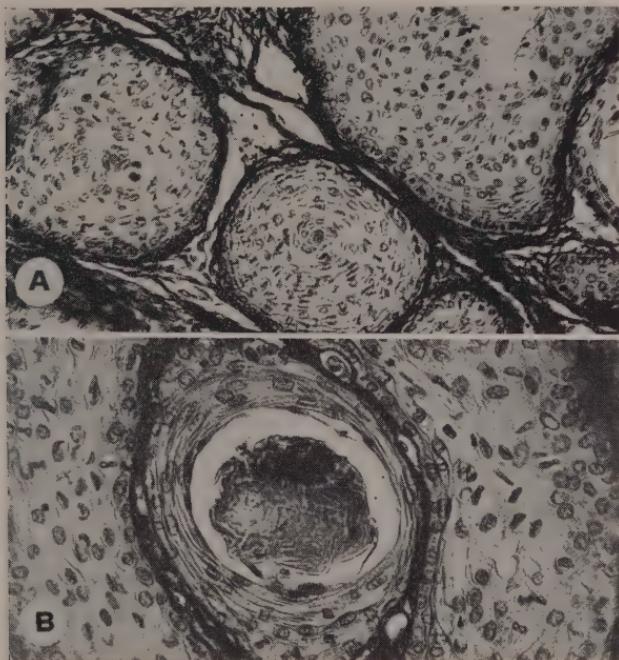


FIG. 95. Details of fig. 94. A. $\times 130$; B. $\times 210$

Burch et al. (1932); my students Osnovikoff (1934) and Viñals (1934); Morató (1941), Nadel (1949). But as already insisted upon results vary greatly as to the degree of the disturbance obtained. It would be difficult to explain these variations. One might assume that the preceding reproductive activity, or inactivity, of the operated animals is implied; one may tentatively suggest that the functional hypophysial changes as induced in the guinea pig by pregnancies or by the prolonged administration of steroids may cause lasting changes in the anterior lobe. Results with the operative interference on the ovary in the rat also seem to vary; but follicular cysts (Wang and Guttman, 1927), and luteal cysts (in our work), were found in the ovarian remnant in rats as in those of guinea

pigs. It was the same, as already stated, in one of two cats on which ovarian fragmentation had been performed (Lipschutz and Voss, 1925). In Rhesus no disturbance of the sexual cycle has been stated in experiments with ovarian fragmentation (Dahl-Iversen et al., 1942). On the contrary, a case of cystic glandular hyperplasia in a woman after removal of one entire ovary and partial removal of the second ovary has been reported (Cruz, 1935). For a discussion of the underlying mechanism of these ovarian changes see chapter 21, B, 1.

It was remarkable that there were no fibroids, uterine or others, in our guinea pigs with ovarian fragmentation, notwithstanding the prolonged follicular phases. But in similar experiments Morató (1941) found one animal with uterine myoma, whereas Nadel (1949) found, also in one animal only, both uterine and mesenteric fibroids. The rare incidence of fibroids may be tentatively explained by the assumption that the occurrence of luteal phases, though in an abnormal sequence, was opposed to production of fibroids. This assumption is substantiated by the presence of corpora lutea, or luteic cysts, in the ovarian remnant, though they may also fail to appear.

Abnormal endocrine ovarian activity similar to that induced by ovarian fragmentation has been obtained also by other experimental procedures as *traumatization of the ovary* by incisions (Wang and Guttmacher, 1927), *painting the ovarian blood vessels with 5 per cent phenol* (Haterius, 1930), and *ligature of the blood vessels* (Fels, 1942) by which vasculation of the ovary is apparently impaired in a transient manner. Under the latter experimental conditions the ovarian endocrine disturbance is, in the rat, probably of even greater consistency than with fragmentation; hypophysial cytology is similar to that after castration (Fels, 1944a). Ovarian and hypophysial changes were produced with a ligature lasting only 30 minutes (Fels, 1947).

A disturbance of the sexual cycle is established in the guinea pig also in *intrarenal ovarian transplantation*; prolonged estrous or long anestrous, cystic glandular hyperplasia of the endometrium and polypous formations have been reported (Lipschutz, 1937b, 1938, with references). A disturbance of the sexual cycle including uterine bleeding has been described in the castrated woman with an ovarian graft; the disturbances may become so considerable that the graft has to be removed (Bourg, 1935). The implication of the hypophysis is rendered very probable by the fact that in the guinea pig blood follicles may appear in intrarenal ovarian grafts (references see p. 177, footnote**) though certainly with a frequency by no means comparable to what is the case with intrasplenic grafts.

An endocrine imbalance has been induced by Pfeiffer in his well known work with *testis grafts in newborn female mice* (Pfeiffer, 1939). In these

animals constant estrous, or prolonged estral phases, sometimes prolonged diestrous, became established. The mammary glands were stimulated. Hypercalcification of the skeleton occurred. In animals with constant estrous the corpora lutea appeared atrophic. The uterus may increase greatly; exceptionally there is pyometra. In animals necropsied at the age of 9 to 13 months cystic glandular hyperplasia of the endometrium was found. The glands penetrated into and through the myometrium reaching and extending out under the serosa. Pfeiffer (1939) compares this atypical behavior in his experimental mice to those in our guinea pigs with ovarian fragmentation. There was in Pfeiffer's mice a certain enlargement of the hypophysis with great increase of chromophobe cells as is customary to find in animals receiving estrogen. Several animals survived over 3 years; among 16 of these animals 4 cases of uterine leiomyoma were present (Pfeiffer, 1949a). In another animal an uterine adenocarcinoma was found with massive metastases in the lymph nodes and invasion of various organs (Pfeiffer, 1949b).

An hormonal imbalance is possibly in play also in castrated mice in which production of multiple lymphomas is enhanced by ovarian grafts. Hypophysial grafts produce a similar result; they act presumably through stimulation of estrogenic secretion by the adrenals (M. Silberberg and Silberberg, 1949b).

Chapter 19.

SUPRARENAL FACTORS OF THE STEROID BALANCE

Under certain experimental conditions estrogen calls for the production of progesterone (p. 181). There is evidence in favor of the assumption that estrogen calls for the production of some substance acting similarly to progesterone also *in the absence of the ovary*. In our experiments with the prolonged administration of fibromatogenic quantities of estrogen to castrated guinea pigs the vagina was found in many cases in a state not of cornification as expected but of partial or complete mucification characteristic of pregnancy, when estrogenic action is antagonized by progesterone. As early as 1930 Del Castillo reported that the estrus condition of the vaginal smear in castrated rats receiving estrogen for several weeks was rhythmically interrupted; he assumed that this was due to rhythmic interaction of a cortical hormone, and subsequently it was discovered that desoxycorticosterone produces an antiestrogenic effect like progesterone (Del Castillo and Di Paola, 1942). Similar observations have been made since 1937 also in the rhesus monkey by Zuckerman, and in further work with rats the conclusion was reached that there is a relation between the sex rhythm and the adrenal cortex (Bourne and Zuckerman, 1940; see also summary of Parkes, 1945). On the basis of our statements one may assume that estrogen stimulates the secretion of antiestrogenic steroids in the cortex for the purpose of adjusting the steroid balance in the body. It is remarkable that progesterone also is produced in the cortex, and one may tentatively assume that part of the antiestrogenic cortical action may be due to this steroid. It may be recalled here that both progesterone and desoxycorticosterone are able to act as antiluteinizers (p. 181).

Desoxycorticosterone and Kendall's dehydrocorticosterone when administered simultaneously with fibromatogenic quantities of estradiol, prevented the production of abdominal fibroids (p. 136). There are no less than thirteen 3-keto-steroids in the suprarenal cortex (Reichstein and Shoppee, 1945), and the question arises whether other cortical 3-keto-steroids also may play any part in the antitumoral autodefense. Experimental investigation in this field is greatly hampered for the moment as these natural steroids are not available in quantities sufficient for work on

afg. actions. Androgens which have been shown to be afg. also are produced in the suprarenal gland.

When making the tentative assumption that cortical steroids partake in antitumoral autodefense because they are afg. one has to question whether the quantities able to prevent estrogen-induced fibroids still are in the physiological range. The question is all the more justified as cortical hormones may produce toxic actions in the body. Kendall (1941) has forwarded the opinion that these toxic actions in patients might be due to the combined administration of large quantities of the hormone and sodium chloride. There was also extensive and fundamental experimental work in this field (Selye and Hall, 1943; Selye, 1947b); toxic actions have been produced in laboratory animals with quantities many times those used in our work. On the other hand, some of our results with guinea pigs are likely to show that afg. quantities of desoxycorticosterone are still physiological and void of any toxic action in this species. We have referred to the fact that suprarenalectomized guinea pigs survive with about 175 μ g of desoxycorticosterone acetate per day absorbed from a subcutaneously implanted pellet (Bruzzone, Schwarz and Borel, 1946). This is more than double what is necessary to prevent estrogen-induced fibroids. Concentration of sodium chloride in the blood was not altered in guinea pigs receiving stilbestrol and 160 to 275 μ g of desoxycorticosterone acetate per day, a quantity which was sufficient to reduce the fibrous abdominal effect from F.T.E. = 5.5 to F.T.E. = 1.7 (Alvarez, 1945; Alvarez and Fuenzalida, 1946).

Most important experimental evidence in favor of the assumption that cortical steroids may partake in an antitumoral autodifensive system is undoubtedly offered by the work of Murphy and Sturm in which the resistance against transmitted leukemia was lowered in rats by adrenalectomy. There was an incidence of 34 to 44 per cent of leukemia in intact rats with an average survival of 9.7 days, as against 77 to 90 per cent in adrenalectomized animals with an average survival of only 6.2 days (Murphy and Sturm, 1943; Sturm and Murphy, 1944). In an other strain of rats normally not susceptible to transmissible leukemia an incidence of 100 per cent with a survival of 6.5 days was obtained after adrenalectomy. The same authorities have described also the preventive action of cortical hormones on transmitted leukemia in rats (see table 15).

Chapter 20.

HEPATIC FACTORS OF THE STEROID BALANCE

We shall deal in this section with another fascinating problem of steroid balance and antitumoral autodefense. It is intimately and primarily related to the part the liver is playing in the metabolism of estrogens whose inactivation in the liver is to-day a well established fact, thanks to the pioneer work of Zondek, Heller, the Biskinds, Segaloff and others (see literature in Jailer, 1948; De Meio et al., 1948; and Mayer and Soumireu, 1948). The problem is of a very wide reach: first, because estrogens are not the only steroids subject to the inactivating action of the liver; and secondly, because quantitative aspects suggest an implication of the liver in bodily homeostasis for the scope of antitumoral autodefense, and of its failure in neoplastic disease.

A. INACTIVATION OF FIBROMATOGENIC QUANTITIES OF ESTROGENS IN THE LIVER

Biskind and Mark (1939) have shown that estrogens absorbed from pellets grafted into the spleen so as to be drained through the portal system were not able to maintain a continuous estrus in castrated rats; when the spleen was sutured to the abdominal wall and the splenic vessels were ligatured, continuous estrus was established. Will the liver be able to protect against fibromatogenic quantities of estrogens absorbed from intrasplenically implanted pellets? Numerous experiments were made with this experimental devise. Pellets of estradiol, estrone and different esters of estradiol as dipropionate and 17-caprylate were implanted into the spleen (L. Acuña, 1942; Lipschutz and Acuña, 1944; Verdejo, 1944; Riesco and Verdejo, 1948). No abdominal fibroids appeared in the course of three months even then when the quantities absorbed were about ten times those necessary to produce fibroids by a subcutaneously implanted pellet. When the pellet began slipping out from the spleen, locating itself partly in the abdominal cavity, fibroids were produced (Lipschutz and Acuña, 1944). This gave additional evidence that the spleen itself was not the inactivating organ.

As we shall see, the protective faculty of the liver against fibromatogenic

quantities of estrogens is quantitatively limited. But our results do not leave the slightest doubt about the fact that under experimental conditions the liver can serve as a barrier against the tumorigenic action of estrogens.

B. COMPARATIVE FIBROMATOGENIC ACTION OF OVARIAN AND URINARY ESTROGENS

It is generally assumed that urinary estrogens, or "metahormones" as we may call them—estriol in the woman, equilin, equilenin and others in the mare—are due to the intrahepatic transformation of ovarian estrogens, or "orthohormones" (estradiol and estrone). Are urinary estrogens still fibromatogenic? Estriol, equilenin and dihydroequilenin have been shown to be able to induce abdominal fibroids (Szabó, 1940b; Thibaut, 1941; Mello, 1944, 1945). But urinary estrogens and their derivatives were, without any exception, less fibromatogenic than ovarian estrogens. Whereas fibroids can be induced with a few μg per day absorbed from subcutaneously implanted pellets of estradiol, 15 to 22 μg of equilenin per day gave an insignificant fibrous effect. Fibroids appeared with 78 μg of equilenin per day. These comparative results suggest that intrahepatic transformation of estradiol, or estrone, into estrogenic metahormones might be highly relevant from the point of view of the supposed antitumoral autodefense.

There was another very interesting finding in the course of this comparative work with ovarian and urinary estrogens. Pellets of estriol, equilenin and dihydroequilenin were implanted into the spleen and allowed to act during 3 months (Becker, 1944; Lipschutz, Becker et al., 1945); the quantities absorbed were similar to those absorbed from subcutaneous pellets. Contrary to our expectation no abdominal fibroids appeared, that is to say, the liver was inactivating these estrogens also (fig. 96). These results show conclusively that transformation of a molecule of an ovarian estrogen into a molecule of a urinary estrogen is not the only mechanism the liver may dispose of for an antiestrogenic or antitumoral autodefense. The question remains open about the special changes the estrogenic metahormones undergo in the liver, that is to say, whether further inactivation is effected solely by conjugation of metahormones with glucuronic and sulphuric acid, or also by transformation into some other substance. In the latter case the number of molecules of estrogen appearing in the urine should be smaller than that set free by the ovary, or administered, for instance, by injection.

C. SPECIAL FUNCTIONAL ASPECTS OF HEPATIC AUTODEFENSE

The above statements make it clear that the ways in which the liver may interfere in the bodily system of antitumoral autodefense are highly

complicated and interwoven. This is evidenced also by what may be called functional limitations and adjustments of the hepatic autodifensive action.

1. Quantitative functional limitations

A quantitative limitation of the faculty of the liver to inactivate steroids has been discovered by Selye and his associates when working on the

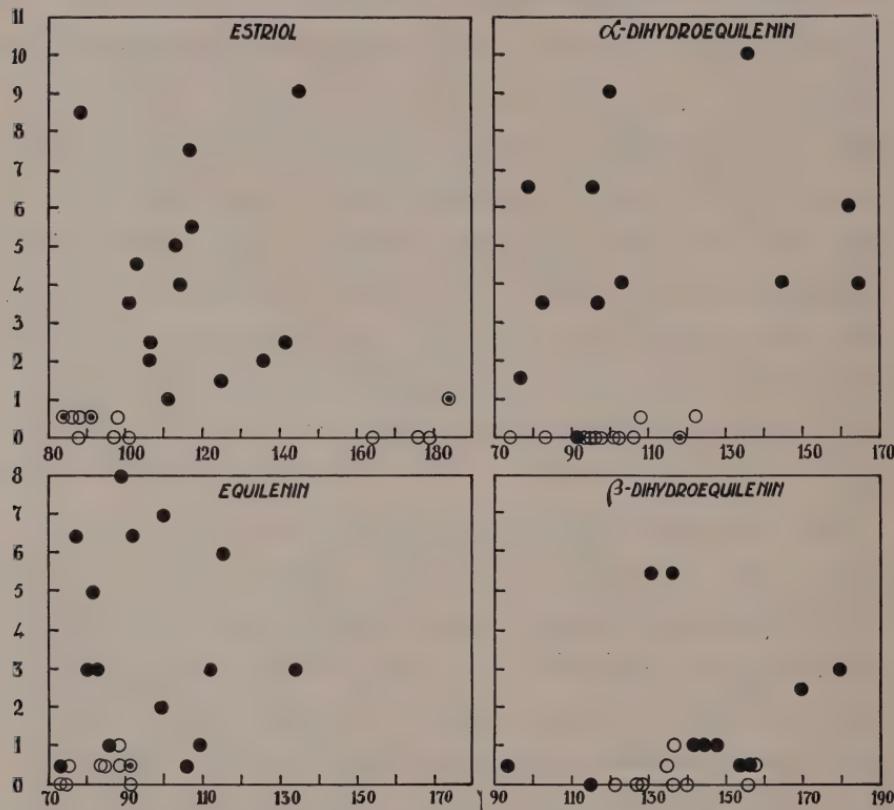


FIG. 96. Comparative results with subcutaneous and intrasplenic pellets of urinary estrogens. Abscissa— μg absorbed per day; ordinates—units of F.T.E. ●, animals with subcutaneous pellets; ○, animals with intrasplenic pellets; ○·, animals with intrasplenic pellets, with adhesions between spleen and abdominal wall. Duration of experiments—3 mo. The diagrams show that urinary estrogens drained through the liver are unable to induce fibroids, that is to say, they are inactivated in the liver.

anesthetic action of these compounds. This action became greatly increased in rats in which the major part of the liver was previously removed (Selye, 1941b). Ablation of 75 per cent of the liver tissue raised the sensitivity 4 to 5 times above that of intact animals; the different steroids are not equally well detoxified (Selye and Stone, 1944).

In our context the quantitative limitation of the inactivating faculty of the liver is of considerable interest. Since the liver is able to inactivate large quantities of estrogen (p. 196), much larger than those necessary to elicit abdominal fibroids, one might, at the first sight, think that there is no quantitative limit as to this. But functional limitation has been demonstrated in experiments in which pellets of estrogen were implanted into the liver. Contrary to what was the case with intrasplenic pellets, fibroids were elicited with intrahepatic ones, though the fibrous tumoral reaction was considerably smaller than with subcutaneous pellets (Carrasco, 1942; Lipschutz and Carrasco, 1944). Estrogen absorbed from an intrasplenic pellet is dissolved in the blood of the splenic vein, and further diluted by the blood of other abdominal vessels joining the portal vein before reaching the liver (fig. 97A). The greatly diluted estrogen is then drained through the entire liver. On the contrary, when absorbed from an intrahepatic pellet estrogen is dissolved in the blood of only a very limited number of interlobular veins (fig. 97B), and reaches the cells but of a *limited hepatic area* through which it is drained; it reaches them in a *concentration too high* to be mastered so completely as in experiments with intrasplenic pellets.

Various other striking examples of functional limitations have been met with. There was, first, the fundamental statement of Segaloff and Nelson (1941) that estrous can be elicited in the rat by intrasplenic injection of all estrogens provided the quantity has been conveniently raised; and secondly, that esterification increases the resistance of estrogen against inactivation in the liver. According to these authorities the estrogenic dose of free estradiol injected into the spleen of the castrated rat is about 50 times that of the subcutaneous dose; with estradiol dipropionate the difference is only about 10 times (see especially fig. 1 in Segaloff, 1949). In our work with fibromatogenic quantities of the dipropionate drained from an intrasplenic pellet through the entire liver they were as fully inactivated as the free hormone. But when the estrogens were drained but through a limited hepatic area the differential behavior of the liver towards free and esterified estradiol became fully evident: with intrahepatic pellets the fibromatogenic effect was more considerable with the dipropionate than with the free steroid (Carrasco, 1942; Lipschutz and Carrasco, 1944).

Artificial estrogens resist inactivation in the body better than natural estrogen (Stroud, 1939). This has been corroborated subsequently also with fibromatogenic quantities of stilbestrol, hexestrol or benzestrol. With intrahepatic pellets of these compounds the fibrous reaction was no less pronounced than with subcutaneous pellets (fig. 98). There were fibroids with stilbestrol and hexestrol even when implanted into the spleen though

the incidence of tumors was greatly diminished (Quintana, 1943; Lipschutz, Quintana and Bruzzone, 1944). The greater resistance was

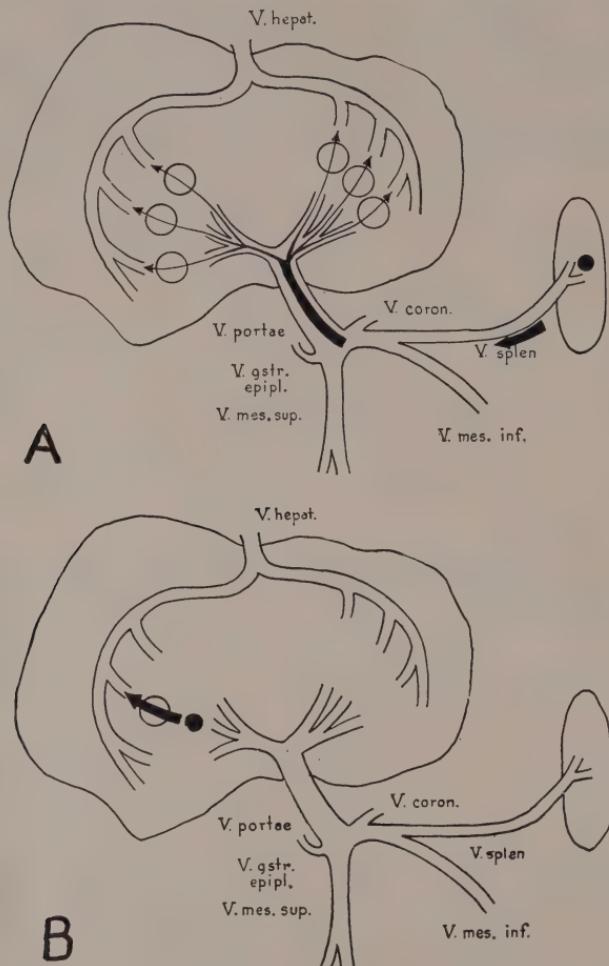


FIG. 97. Comparative extent of hepatic contact of the hormone absorbed from intrasplenic and intrahepatic pellets. A. *The hormone absorbed in the spleen reaches the liver after having been diluted in the venous blood of the whole spleen and probably also in the blood from other abdominal sources. The hormone is distributed in the whole liver thanks to the branching of the portal vein.* B. *The hormone absorbed in the liver is diluted in the blood but of a limited number of lobular veins and is consequently carried on in a relatively high concentration to a restricted hepatic area.*

evidenced also by other toxic actions as genital bleeding, uterine growth, endometrial polyps, great development of the nipples and mammary glands. These actions were absent with intrasplenic pellets of natural

estrogens, or appeared only exceptionally and transiently; they were always present with intrasplenic pellets of artificial estrogens. Greater resistance of artificial estrogens against intrahepatic inactivation has been reported also by other authorities (Segaloff, 1943; Zondek et al., 1943). The behavior towards bisdehydrodoisynolic acids is of an especial interest; it will be dealt with in paragraph 2.

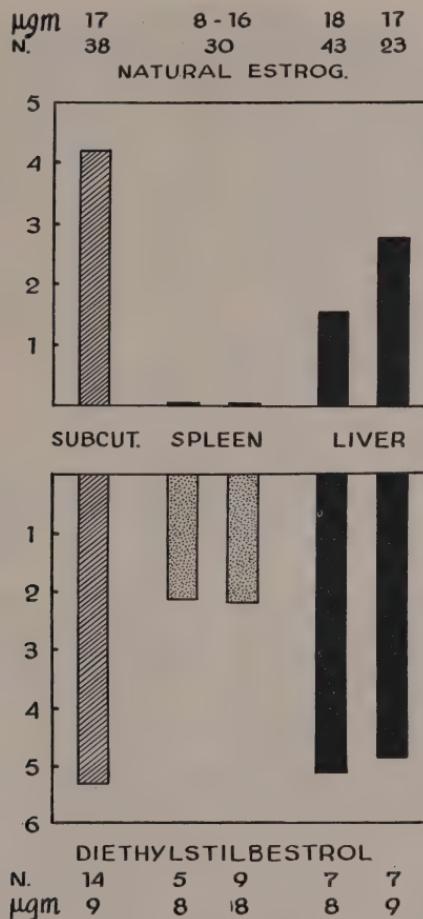


FIG. 98. Differential behavior of the liver towards natural and artificial estrogens. Average fibrous tumoral effect in 42 animals with pellets of stilbestrol, and in 143 animals with pellets of natural estrogens (estrone, estradiol, and different esters of estradiol). Duration of the experiment—70 days. Quantities absorbed per day in μg ; N—number of animals in each group. The second column with intrasplenic or intrahepatic pellets gives the results with animals with adhesions to the abdominal wall. The diagram shows that with intrasplenic pellets of stilbestrol inactivation was not complete; with intrahepatic pellets of stilbestrol the fibromatogenic action was almost the same as with subcutaneous pellets. The adhesions were evidently of no quantitative importance in these experiments.

The quantitative limitation of the hepatic antiestrogenic activity has been demonstrated also in our work with comparative intrasplenic and intrahepatic autoplasic ovarian grafts. The latter behave quite differently than the first (Ponce de León, 1944; Lipschutz, Ponce de León et al., 1946). There was considerable development of the nipples, mammary

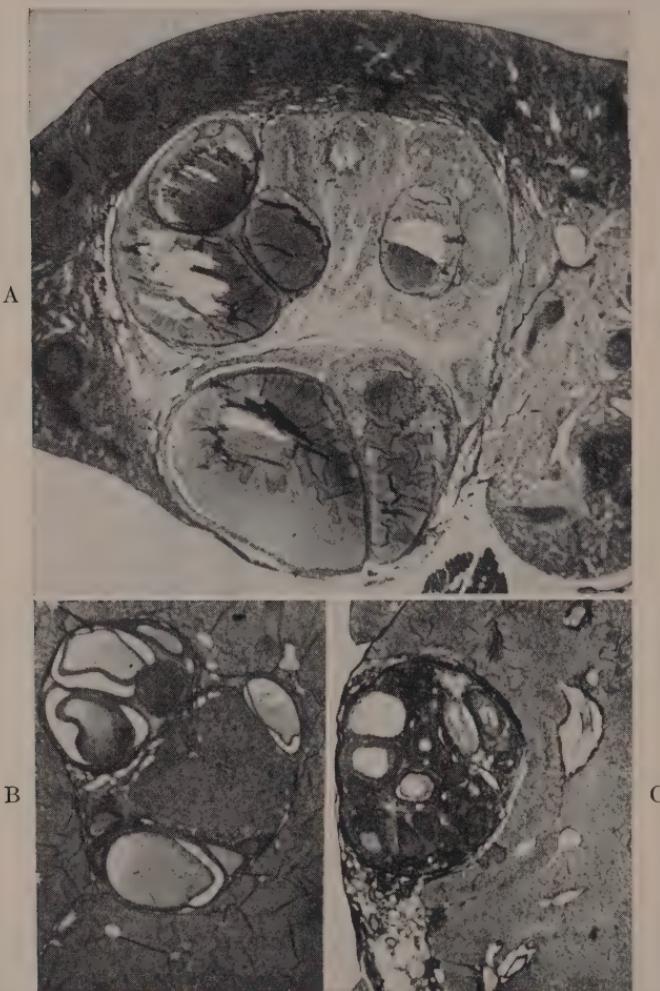


FIG. 99. Comparative behavior of intrasplenic and intrahepatic autoplasic ovarian grafts in castrated g. pigs. A. Intrasplenic, 98 days after grafting. Numerous hemorrh. follicles; several of them cystic. $\times 10$. (LII.55). B. Intrahepatic, 146 days. Many Graafian follicles, but absence of hemorrh. foll. Corpus luteum. This was the largest intrahepatic graft. $\times 10$. (LII.29; erroneously given as LII.49 in fig. 15 in Rev. Canad. Biol. 5: 181.) C. Intrahepatic, 177 days. Graafian foll. Absence of hemorrh. foll. $\times 10$. (LII.4).

glands and the uterus which does not occur with intrasplenic grafts. Inactivation of estrogens drained from an intrahepatic graft was consequently less complete than with intrasplenic grafts. There were no blood follicles in the intrahepatic graft and it remained small (fig. 99). The microscopical aspect, though variable, is in most of the cases coincident with that of a normal ovary (fig. 88A).

2. Functional adjustments

The concept of quantitative adjustments of the hepatic function of antiestrogenic autodefense is based on various experimental observations. Those of Biskind (1941) have to be mentioned in the first place. When a pellet of estrogen was implanted into the spleen of the castrated rat estrous was produced which lasted 2 weeks. Only subsequently a nonestrus period was established which lasted weeks or months, that is to say, for the rest of the experiment, though the pellet was always present in the spleen. This sequence of transitory estrous and permanent anestrus might be explained by the assumption that the faculty of the liver to inactivate the steroid has been stimulated by the latter. Decrease of absorption from an implanted pellet is in two weeks not sufficiently conspicuous so as to explain the consecutive anestrus. Our work with the absorption of fibromatogenic quantities from intrasplenic pellets in castrated female guinea pigs is strongly in favor of a functional adjustment. The vaginal opening was maintained open in these guinea pigs, as in Biskind's rats, for several weeks and sometimes even more, especially with the esterified hormone (L. Acuña, 1942). Subsequently the vagina became closed and remained so till the end. In one of our animals with the vaginal introitus closed cystic glandular hyperplasia of the uterus was found (fig. 100). It was evidently produced before the functional adjustment was completed. But the cells lining the cysts were small and poor in protoplasm like endometrium undergoing involution after the withdrawal of estrogen; this happened evidently in the weeks following adjustment. It is not very probable that absorption dropped suddenly so greatly as to explain the sequence of the two phases of estrous and anestrus.

Selye (1941c), in his work on the anesthetic action of steroids, has stated that resistance against these steroids, and so also against stilbestrol, increases when quantities insufficient to cause the anesthetic effect are given previously during 10 consecutive days by intraperitoneal injections. Zondek et al. (1943) found that the capacity of liver pulp to inactivate stilbestrol increased when the rats were pretreated with this estrogen.

Adjustment of the liver to increased demands related to the metabolism of steroids, one of the most striking examples of biochemical adaptation in the realm of highly specialized bodily functions. It might be compared to

the enzymatic adaptation in microorganisms which, when placed in contact with some substance, acquire the enzymes necessary to metabolize it (summary and references Spiegelman, 1948, p. 292.)

The behavior of an artificial estrogen, the bisdehydrodoisynolic acid (BdD.a.), and its derivatives, in the liver offers considerable interest. Segaloff (1948a) made the remarkable discovery that the estrogenic activity of sodium bisdehydrodoisynolate and of the 3-methyl-ether of BdD.a. is, in the castrated rat, even enhanced in its passage through the liver. The interesting artificial estrogen of Horeau and Jacques, the dimethyl-ethyl-allenolic acid, is by intrasplenic injection in the castrated rat as estrogenic as when injected subcutaneously (Courrier, Horeau and Jacques, 1947). When pellets of the 3-methyl-ether of BdD.a. or of the methyl-ester of the latter were implanted into the spleen the nipples and

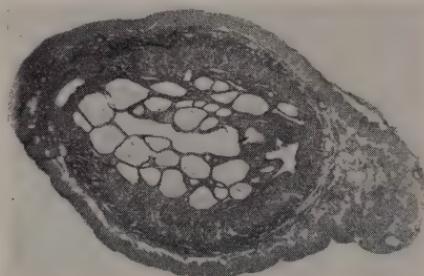


FIG. 100. Functional limitation of the inactivating faculty of the liver. Cystic glandular hyperplasia elicited by intrasplenic pellet of estrad. 16 μ g of estrad. daily, 70 days. The vagina was open during the first three weeks when it closed and remained so for the rest of the experiment; but the glandular cysts have persisted. $\times 10$. (XLII.3).

mammary glands developed, the vaginal entrance was maintained open for 3 months; there was uterine bleeding, the uterus greatly increased, cystic glandular hyperplasia and metaplasia were produced and the proliferated glands invaded the myometrium; sometimes fibroids were produced (Iglesias, Lipschutz and Mardones, 1950b). Both derivatives of the BdD.a. when absorbed from the spleen were in any case as active as when absorbed from beneath the skin. No functional adjustment of the liver occurred in the course of 3 months.

There is also the question how far transitory storage of estrogens in the bile as evidenced by the work of the Cantarow group interferes in the fate of these compounds in the liver. Even relatively large quantities of intravenously injected estrogen are stored in the bile of the dog (Cantarow et al., 1942a, 1943a) but also of the woman (1943b); as much as about 90 to 95 per cent of α -estradiol administered may be found in the course

of the next 48 to 72 hours in the bile of the dog. With stilbestrol the faculty of storage is effected at a slower rate (Cantarow et al., 1942b). There is storage also of endogenous estrogen as can be shown in women at full term pregnancy or various days after delivery, or in the dog with the administration of gonadotrophins from pregnant mare serum (Cantarow et al., 1943b). Estrogen goes to the bile even when absorbed from pellets of estradiol implanted into the spleen (Cantarow et al., 1943b). It is evident that the timing of intrahepatic inactivation of estrogens must be profoundly influenced by the storage mechanism reported by the Cantarow group.

D. INTRAHEPATIC INACTIVATION OF PROGESTERONE, DESOXYCORTICOSTERONE, AND ANDROGENS

Inactivation of estrogens in the liver will easily be appreciated as a fundamental means of bodily autodefense against a growth factor which under certain quantitative and timing conditions may assume toxic and tumorigenic faculties. More intricate, though only at first glance, the fact may seem that steroids which have been recognized as antitumorigenic also are inactivated in the liver. This refers to progesterone and desoxycorticosterone.

There is full evidence that *progesterone* is inactivated in the body to be transformed into pregnanediol which is excreted with the urine (see full references to the work of Zondek, Hamblen et al., Venning, Brown Hoffman, Dorfman et al. in Masson and Hoffman, 1945; Marrian, 1949). But there were at the beginning contradictory statements as to where this inactivation is effected. Mussio-Fournier, Buño and Morató-Manaro (quoted from Albrieux et al., 1940) were probably the first to reach the conclusion that inactivation takes place in the liver because the progestational threshold of progesterone was the fivefold when injected into the spleen and liver or intraperitoneously instead of being injected subcutaneously. Fels and Mónaco (1941) did not corroborate this finding, and various workers failed to observe inactivation of progesterone by liver *in vitro*. But as early as 1941, Selye (1941b) demonstrated that the anesthetic action of progesterone administered by intraperitoneal injections in the rat is considerably enhanced by partial removal of the liver (see also p. 198 with reference to steroids in general). Since 1944 new conclusive statements on intrahepatic inactivation of progesterone were made by various authorities. In our work evidence was given that quantities of progesterone sufficient to prevent estrogen-induced fibroids in the guinea pig when absorbed from subcutaneous pellets failed to do so when absorbed from intrasplenic pellets (Dosne, 1944). With larger quantities of progesterone the afg. activity became again manifest. It was the same with

progesterone absorbed from pellets implanted into the liver (Bruzzone and Cuevas, unpubl.). It seems evident that the inactivating faculty of the liver is, with progesterone as with estrogen, quantitatively limited. Kochakian et al. (1944) demonstrated the inactivation of progesterone in the liver of the rabbit. Masson and Hoffman (1945) showed that the pregestational threshold of progesterone which when administered by subcutaneous injection was of 0.25 mg, increased to 200 mg when given by gavage; but when partial hepatectomy was made prior to administration of progesterone the threshold decreased to as little as 25 mg. With the work of Masson and Hoffman the question of inactivation of progesterone in the liver seems to be settled.

There were at the beginning contradictory statements also on the behavior of the liver towards *desoxycorticosterone*. No significant difference was found in survival of suprarenalectomized rats with intrasplenic and subcutaneous pellets of desoxycorticosterone acetate (Eversole and Gaunt, 1941); the decrease of sodium chloride was apparently the same (Mark, 1942). But other authorities reported that with intramesenteric pellets sufficiently small intrahepatic inactivation became manifest, and that complete inactivation failed only when larger pellets were used; they concluded that the faculty of the liver to inactivate the cortical steroid is quantitatively limited (Burril and Green, 1948; see also Greene, 1948). The antifibromatogenic activity of desoxycorticosterone was abolished when absorbed from intrasplenic pellets (Dosne, 1944).

One wonders what the significance of special devices for the inactivation of such steroids as progesterone and desoxycorticosterone may be. From the point of view of a steroid homeostasis for antitumoral autodefense such devices seem contradictory. But on the other hand, there is evidence that progesterone, like estrogen, may produce under certain timing conditions an alteration of the ovarian rhythm. Progesterone is apparently fundamental for the steroid control of the sequence of the ovarian phases (p. 181). The hitherto prevalent idea that estrogen is the principal or only steroid which partakes in the control of ovarian-hypophyseal functional complex seems to have been a failure. Progesterone and desoxycorticosterone have been shown to be potent antiluteinizers. One may reasonably assume that the latter function also needs to be controlled as to intensity and time so as to allow for the normal ovarian rhythm. We know indeed very little about the part which the quantitative and timing interrelations between estrogenic and antiestrogenic steroids have in the establishment of the normal rhythm, and about the part played therein by the liver.

Androgens also are inactivated in the liver as has been shown by the Biskind group (G. R. Biskind and Mark, 1939; M. S. Biskind and Biskind,

1943), and studied recently in a more detailed manner especially by the groups of Dorfman and of Samuels (see Samuels et al., 1947; Samuels, 1949).

E. IMPAIRMENT OF THE HEPATIC FUNCTION AND CANCER

Experimental evidence in favor of a steroid homeostasis as an autodefensive antitumoral device, and of the liver being one of its important parts, is overwhelming though many details cannot as yet be defined. Can these experimental findings be applied to neoplastic disease in man, and to therapeutics? In any case must one admit that the comparative study of the metabolism and excretion of steroids in healthy and cancerous individuals may be considered as one of the essentials of modern cancer research (p. 103). Thus a fundamental aspect of clinical cancer research is intimately related to the liver: impairment of hepatic function as a possible factor of a disturbed steroid homeostasis and cancer.

A direct approach to the question has been made by Twombly and Taylor (1942). They found no difference between healthy individuals and cancer patients in the capacity of their liver to inactivate estrogen *in vitro*. Similarly, the inactivating faculty of the liver of four different strains of mice was not correlated with their susceptibility to spontaneous mammary cancer. However, on the other hand, a considerable wealth of indirect experimental approaches related to the above question has been accumulated in recent years.

Inactivation of endogenous or administered estrogen has been shown to lessen when the liver was damaged experimentally by carbon tetrachloride (Talbot, 1939; Pincus and Martin, 1940; recent remarkable work of Furlong et al., 1949) or after partial hepatectomy (Schiller and Pincus, 1944). Inactivation of estrogen by the liver is apparently a very complex phenomenon; the conflicting result has been reported that livers of rats poisoned with carbon tetrachloride had *in vitro* the same inactivating faculty as normal liver (Cantarow et al., 1943a). Great attention was attracted by the finding that in rats placed on a vitamin B complex free diet, estrogens produced in an intrasplenic graft, or absorbed from an intrasplenic pellet, pass through the liver without being inactivated; the impairment presented itself already at a minor degree of vitamin B deficiency; addition of brewers yeast was curative (M. S. Biskind and Shelesnyak, 1942; M. S. Biskind and Biskind, 1942). Thiamine and riboflavin were reported to be the factors in play (Segaloff and Segaloff, 1944). Subsequently these conclusions were questioned. Zondek and Finkelstein (1947) stated that only 10 per cent or less of estrone injected into vitamin B deficient rats could be recovered five hours later from the minced bodies; but the liver of simi-

lar animals was found to be unable to inactivate estrone in vitro. It has also been reported that the hepatic functional failure was due not to vitamin B deficiency but to low protein diet (György, 1945), or to inanition concomitant with the B deficient diet (Drill and Pfeiffer, 1946). The antiestrogenic function of the liver may be impaired in spite of large amounts of thiamine and riboflavin when food intake is restricted (Jailer, 1948). But whichever the direct cause of this experimentally induced failure of the liver may be, there is the fact that a steroid imbalance can be produced by an impairment of the liver through the diet.

Various authorities have emphasized the clinical relevance of the above experimental statements. In men with cirrhosis of the liver testicular atrophy, gynecomastia, loss of libido, decrease of body hair, female distribution of pubic hair have been reported. Increase of unconjugated urinary estrogen was found associated with testicular atrophy and gynecomastia (Glass et al., 1940). When estrogen is injected in men with cirrhosis the quantity of excreted estrogen increases very considerably (Glass et al., 1944), as compared with normal individuals (Pearlman and Pincus, 1943; Schiller and Pincus, 1943). There is an increase in the yield of urinary estrogen also in males with infectious hepatitis (Gilder and Hoagland, 1946). Uterine bleeding occurs in women with cirrhosis of the liver, and menstrual disturbances have been reported in intoxication with liver poisons such as lead or others.* Menorrhagia, cystic mastitis, premenstrual tension, and probably uterine myoma have been reported as due to failure of the liver to inactivate estrogen owing to the B vitamin complex deficiency (M. S. Biskind, Biskind and Biskind, 1944). The clinical importance of these statements is apparently all the greater as the liver, even when no more able to inactivate estrogens, continues in the B deficient rat to inactivate androgens (M. S. Biskind and Biskind, 1943) which are known to counteract the toxic and neoplastic actions of estrogen. Prompt responses were obtained in the described pathological conditions with a vitamin B treatment (M. S. Biskind and Biskind, 1944).

Canadian authorities have recently added important new data suggesting that thiamine deficiency may be implicated in uterine cancer in women (Ayre and Bauld, 1946; Ayre, 1947). In cancer of the cervix abnormal estrogenic activity estimated by the vaginal smear cytology was coupled with thiamine deficiency. In senile patients with cancer of the cervix endometrial hyperplasia also may be found. Thiamine deficiency occurred in 86 per cent

* Cirrhosis of the liver was found at autopsy in 4 out of 12 women with primary carcinoma of the endometrium but in only 1 out of 54 women with metastatic carcinoma in the uterine fundus (Speert 1949).—For more references see Burrows (1949), p. 310 and 322.

of cases with cancer of the cervix, as against 10 per cent in controls. The thiamine excretion level may drop to less than half the average level in healthy women (Ayre, 1947; see also critical review of Greene and Peckham, 1947). Riboflavin deficiency also has been reported to be in play (Ayre, 1947).

These clinical statements will convince that experimental results on which the concept of an antitumoral autodefense through an adjustment of the steroid balance is based, deserve utmost interest in clinical research.

Chapter 21.

NEOPLASTIC GROWTH OF TESTICLE, OVARY AND ADRENALS INDUCED BY OVERTHROW OF THE GONADAL-HYPOPHYSIAL RELATIONSHIP

A. ATYPICAL GROWTH OF THE INTERSTITIAL CELLS OF THE TESTICLE

In work with operative interferences on the testicle and the ovary, and subsequently with intrasplenic ovarian grafts, different aspects of atypical growth have been discovered. Nodular growth of the interstitial cells occurs sometimes in small testicular remnants in the guinea pig ("testicular fragmentation"; Lipschutz, 1924; p. 151). These remnants of the upper pole of the testicle are splendidly vascularized by branches of the upper spermatic artery. In remnants staying in the abdominal cavity the seminiferous tubules undergo degeneration, or what may be better called "backward development." Whereas the tubular wall may be reduced to a layer of Sertoli cells, there is great development of interstitial tissue; the cells increase in size and number. They may be found in irregular clusters; exceptionally nodules as in fig. 101 may be found. The nucleus of these cells is of a variable and sometimes considerable size (fig. 101B). The clusters or nodules derive, apparently, by proliferation from normal or hypertrophic interstitial cells. But groups of cells or small clusters which form part of a nodule, may be surrounded by a conjunctive membrane (our work with Sanhueza, 1932); this microscopical picture suggested that they may originate partially from the proliferating and hypertrophying cells of the membrana propria of the degenerating tubules (E. Aron, 1931), and that these cells become incorporated into the interstitial tissue.

It is now known that the interstitial endocrine gland of the testicle is stimulated by a gonadotrophic hormone seemingly coincident with the LH of the anterior lobe of the hypophysis. One may then assume that atypical proliferation of interstitial cells is due to an overthrow of the gonadal-hypophysial relationship, in analogy to what takes place with ovarian fragmentation (see chapter 18, p. 190). But the condition of the testicular remnant is, in the guinea pig, not always similar to that described above, especially when it remains in the scrotum and not in the abdominal cavity; then the increase of the interstitial tissue is much less pronounced

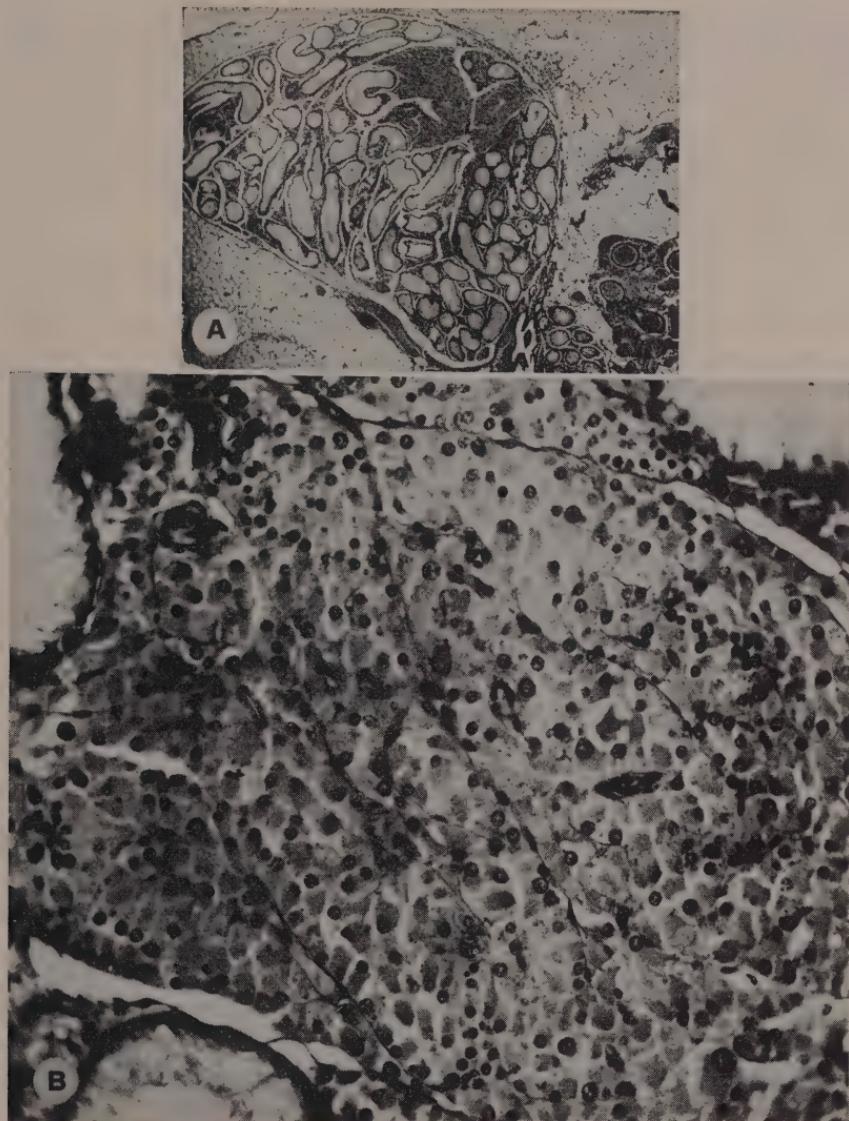


FIG. 101. Nodular increase of interstitial tissue in testicular remnant of g. pig, 135 days after removal of the left testicle and of the greater part of the right one. Remnant embedded into the fatty body of the testicle; its weight about 20 mg, or 5 per cent of the normal weight of two testicles. To the right of the remnant—head of the epididymis and plexus pampiniformis (D.30). A. Big nodule of interstitial tissue surrounded by degenerated seminiferous tubules. $\times 17$. B. Part of the nodule at higher magnification. Variation of nuclear size of interstitial cells mostly hypertrophied. $\times 220$.

(Lipschutz, 1924, 1926). In testicular remnants in mice and in the pigeon we saw no increase at all. One must assume that non-specific stimuli, *besides* the hypophysial LH, may be in play in the hyperplastic or tumoral response; one of these unspecific stimuli is most probably vascularization on which much—or too much—stress has been laid by myself in former years (1922, 1924); or the heat due to location in the abdominal cavity as stated by C. R. Moore (1939).

Greatest interest deserve the statements about tumoral responses of the interstitial tissue, elicited by estrogen in mice. An increase of interstitial tissue due to hypertrophy and proliferation of cells has been produced in this species by the action of estrogens already many years ago (Burrows, 1935, 1936b; Gardner, 1936, 1937; quoted from 1947b). It has been also reported as early as 1937 that under these experimental conditions the testicle may consist almost exclusively of interstitial tissue, the testicle attaining, or surpassing, its normal size notwithstanding the seminiferous tubules being highly reduced (Burrows, 1937). Nodular tumors of the interstitial tissue were discovered by Bonser and Robson (1940) when working with Strong's A strain and using triphenylethylene as estrogen. All animals surviving 50 weeks of treatment had testicular tumors, and metastases in the lung were detected between the 67th and 75th week (Bonser, 1942). Tumors were found only in certain strains and not in others where rapid atrophy of the testicle took place under the influence of estrogen (Bonser and Robson, 1940). The interstitial cell tumor has been found also by other workers (Hooker et al., 1940; Shimkin et al., 1949; Hooker and Pfeiffer, 1942; Gardner, 1943a). These tumors when transplanted may grow without estrogen being administered (Bonser, 1944). In other strains estrogen must be given so that the transplant may grow; it is very remarkable that a tumoral transplant may lie dormant for several months to start again growing when estrogen is given (Gardner, 1945).

These tumors of the interstitial tissue in mice are most probably due to the stimulative action of estrogen on the anterior lobe which then delivers great quantities of LH. Similar tumors were induced in mice by the prolonged administration of the serum from the pregnant mare (Pfeiffer and Hooker, 1943).

Estrogen-induced tumors of the interstitial tissue have not been reported in other species. They failed to appear in rabbits even in experiments of long duration (Chevrel-Bodin and Leroy, 1941). In the guinea pig administration of estrogen (stilbestrol and hexestrol) has been prolonged for as much as 8 months. There was a decrease of the interstitial tissue in about half of the treated animals (fig. 102A and B) (Echániz, 1944). These results may be due to the time of treatment not having been sufficiently long. But the treatment was sufficient to cause a very considerable inhibition of body growth (fig. 102C). It would seem likely that the tumoral

response to estrogen is dependent on the inherited condition of the territory on which the hormone is acting; but even then the question would remain open whether the result so different according to the species is dependent

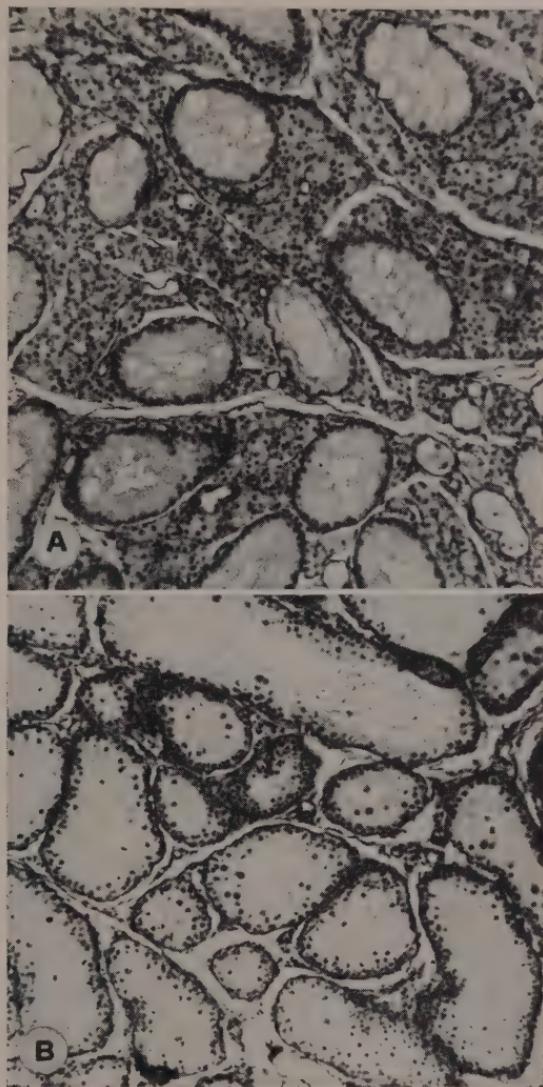


FIG. 102A AND B

on an inherited differential response of the hypophysis or of the testicle. The latter seems more probable as estrogen stimulates in the guinea pig production or release of the luteinizing hormone (p. 181).

There is production of androgen in experimentally induced interstitial

cell tumors. Resistance to certain actions of estrone, most probably due to androgen produced in the interstitial tissue, has been reported in a male mouse with estrogen-induced interstitial cell hyperplasia, or tumor; whereas there were separation of pubic bones and adrenal changes as usual with estrone, the male accessory organs were microscopically normal and contained secretion (Burrows, 1937). The excess of androgen protects against squamous metaplasia of the coagulating glands (Armstrong and Bonser, 1948). This type of "acquired" resistance may be taken as a suggestive example of those extraordinary autodifensive hormonal means the organism may dispose of in cases of emergency—though certainly failing in the end, under these experimental conditions!



FIG. 102C

FIG. 102. Inhibition of interstitial tissue by estrogen in g. pig. A. Testicular remnant, 230 days after operation. Great development of interstitial tissue as in fig. 101. $\times 90$. (LIII.66). B. Testicular remnant, 227 after operation, 217 after implantation of pellet of stilbestrol. $\times 90$. (LIII.7). Absence of interstitial cells. C. Inhibition of body growth by estrogen: in the center the same animal as B; to the left—male of the same litter, with testicular remnant but without estrogen; to the right—normal male of the same litter.

The implication of a hormonal imbalance in testicular lesions has been assumed also on the basis of the statement that they can be found in strains of mice with spontaneous mammary cancer (Athias and Furtado-Dias, 1947).

Spontaneous tumors of the interstitial tissue have been found in the dog where they are frequent, in the horse, and probably also in mice (references Zuckerman and McKeown, 1938; Bonser and Robson, 1940; Innes 1942; recent summaries Willis, 1948, p. 95, 555, 580; Nelson, 1948). In man interstitial cell tumors are only 1 per cent of testicular neoplasms, according

to a report on 922 cases (Friedman and Moore, 1946; Lewis, 1948). Most of these tumors grow in man slowly and in general do not produce metastases; but the latter also has been reported (Weil, 1936; Willis, 1948, p. 580). These interstitial cell tumors in man produce androgen; there may be precocious development of the sex characters which regress after removal of the tumor (Willis, 1948, p. 582). Gonadotrophic hormones have not been found in the urine (references Sandblom, 1948).

The nodular increase of interstitial tissue similar to that found in retained testicles in various animals (pig, dog; Innes, 1942) and in man occurs also in "women" and then deserves special clinical interest (Cádiz and Lipschutz, 1933; Bettinger, 1944; fig. 103). Whether these testicular structures in women are to be compared functionally to the interstitial cell tumor in man, cannot be said with certainty; the retained testicle in women seems to be able to produce both female and male hormones the first prevailing in one case, the second in another (see various new cases in Salaber et al., 1949). Masculinizing Leydig cell tumors in the ovarian hilus and the mesovarium also have been reported (Sternberg, 1949). But hyperplasia of the endometrium of the senile uterus, i.e. a supposedly estrogenic action, also has been attributed to peculiar groupings and clusters of cells in the ovarian hilus (Husslein, 1948).

Other spontaneous testicular tumors (Friedman and Moore, 1946; Scully and Parham, 1948)* also deserve great interest in our context, because they are often accompanied by the excretion of hypophysial gonadotrophic hormones denoucing by this an implication—primary or secondary—of the anterior lobe (Weil, 1936, p. 125–140; Hamburger, 1938, 1941, references; Hamburger and Godtfredsen, 1941; Twombly et al., 1942; Twombly, 1944). Various experimental results with the production of seminoma and teratoma may be explained by the assumption that they were due to an overthrow of the hormone balance between the testicle and the anterior lobe. Thus seminoma has been produced in the rabbit by intratesticular injection of equilin in oil (Burrows and Horning, 1947), and in fowl by partial removal of the testicle (Champy and Lavedan, 1939). Michalowsky, since 1928, reported production of teratoma with injections of zinc into the testicle of fowl; his statements have been amply confirmed by Bagg and others (references Falin, 1940; Berrill, 1943; Willis, 1948, p. 974).

Vasquez-Lopez (1944) found carcinoma in the head of the epididymis in the hamster treated during 10 months with estradiol.

Spontaneously occurring Sertoli cell tumors offer great interest: they produce feminizing hormones and not masculinizing ones (Zuckerman and

* For classification of testicular tumors see also Foot (1949) and Moon and Hullinghorst (1949).

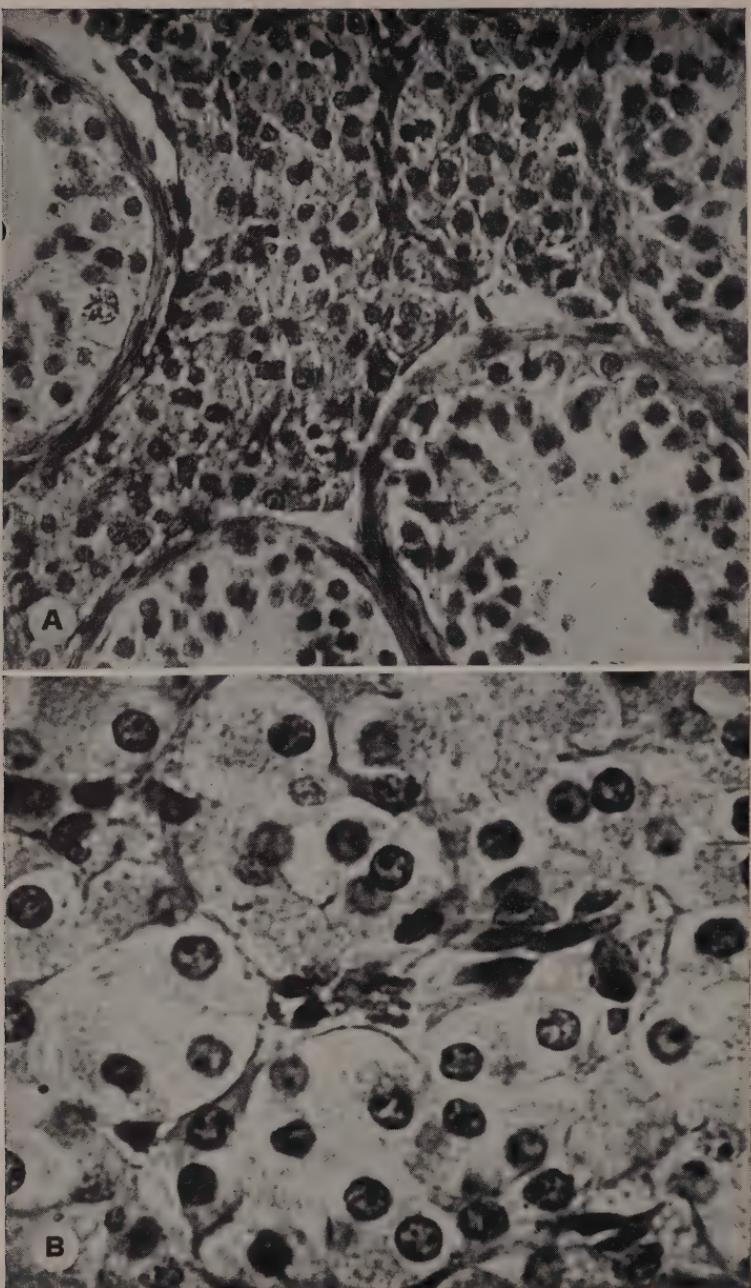


FIG. 103. Retained testicle in "woman." A. Nodular development of interstitial tissue surrounded by seminiferous tubules with incomplete spermatogenesis (there were spermatocytes). $\times 290$. B. Same testicle. Cords of interstitial cells beneath the albuginea. Groups of cells are kept together by collagenous membrane. Abundance of capillaries. $\times 870$.

McKeown, 1938; Huggins, and Moulder, 1945; Berthrong et al., 1949), and these feminizing hormones may act on the breasts and the prostate (see also the work of Albright and ass., of Del Castillo et al., 1947, and Teilum, 1949).

B. ATYPICAL GROWTH OF OVARIAN TISSUES

1. *Follicular and luteic cysts*

When an ovary is grafted into the kidney of a castrated male guinea pig corpora lutea fail to be produced; follicles attain the size of mature structures and they persist in this condition. "Hyperfeminization" of the male, as known since the work of Steinach, has been shown to be correlated in the guinea pig with this "cystic" persistence of follicles (Lipschutz, 1927). Greater deviation from the normal can be found, as already mentioned (p. 190), in small ovarian remnants left in the body after the removal of one entire ovary and the greater part of the second; luteic cysts have been described in the cat (fig. 104), in the rat, in the guinea pig (fig. 105) (Lipschutz, 1931, 1937b, 1938). Exceptionally a luteic cyst is filled with blood (fig. 106). Similar hemorrhagic luteic cysts occur also in intrasplenic grafts in female guinea pigs (Lipschutz, Ponce de León et al., 1946). The latter finding gives additional evidence that this pathological condition of the remnant is due to an overthrow of the ovarian-hypophysial relationship, the normal functional sequence of, or the normal balance between, the two gonadotrophic factors, the FHS and LH being disturbed. The finding of Smith and Engle (1927) that in adult rats luteic cysts are produced with the administration of anterior lobe strongly favors the above conclusion.

Why the normal ovarian-hypophysial relationship should be overthrown by ovarian fragmentation? The relevance of this question is all the greater as so much evidence has been given that the overthrow may cause atypical growth both in the ovaries and the genital tract. It does not seem very likely that the overthrow were due, in ovarian fragmentation, solely to a failure of the steroid control of the hypophysis. The ovarian remnant not only continues producing estrogen; follicular phases are often even prolonged and production of estrogen is probably also increased. There must then be some other mechanism of control which has failed. We have already referred (p. 182) to the old concept that there is a balance between production of extraovarian X-substances necessary for follicular development—to-day hypophysial gonadotrophins—and their consumption in the ovary, and that it is this balance which is overthrown in ovarian fragmentation (Lipschutz, 1928). This concept was substantiated also by the statement that when one ovary is left at its normal place together with a remnant of the other ovary no changes occurred in the ovarian structure and the

genital tract; that is to say, the intact ovary protects against any alteration of the ovarian-hypophysial endocrine function (though the protection afforded is possibly not a complete one; C. Aron and Marescaux,



FIG. 104. Ovarian remnants under different experimental conditions in the cat. A. Luteic cyst in greatly increased remnant alone present in the body, $5\frac{1}{2}$ mo. after operation. Various cystic follicles, $\times 23$. (D.309). B. Same. Luteinized wall of cyst. $\times 100$. C. Ovarian remnant of cat, $5\frac{1}{2}$ mo. after operation; the rest of the ovary and the entire second ovary also were present. No increase of remnant. Great number of primary follicles. $\times 23$. (D.311).

1948). Or, in terms of the balance between production and consumption of gonadotrophins: though the number of follicles which ripen in an ovarian

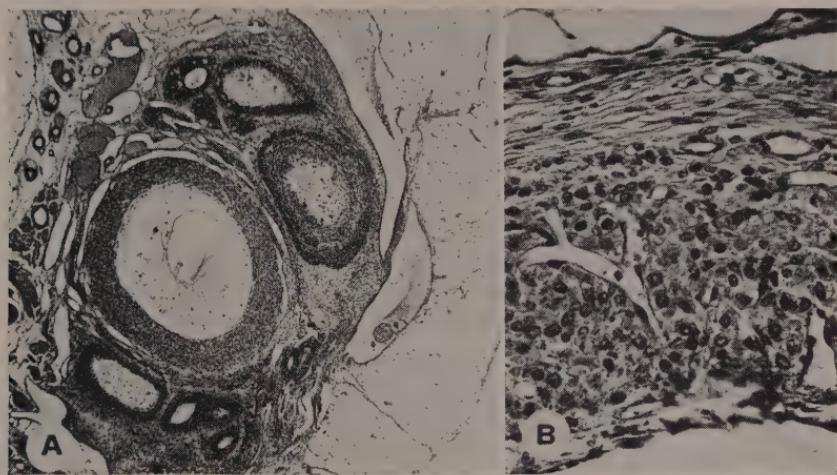


FIG. 105. Luteic cysts in ovarian remnants in g. pig. A. 79 days after operation. Adipous tissue. Germinative epithelium. There was a second luteic cyst not seen in the section. $\times 22$. (Ch.753). B. Same. Luteinized wall of the cyst. $\times 210$.



FIG. 106. Hemorrhagic luteic cyst in ovarian remnant in g. pig. 59 days after operation. $\times 26$. (Q.L.12).

remnant, when alone present in the body, is no less than in two normal ovaries—in compliance with the law of follicular constancy (Lipschutz,

1927, 1937b, 1938)—the ovarian-hypophysial balance is overthrown; but there is no overthrow of the balance when the ovarian remnant is accompanied by an intact ovary at its normal place. That protection afforded by the latter, against the overthrow through ovarian fragmentation, is effected not by the steroid control but by consumption has been substantiated by experiments in which the entire ovary, accompanying the small ovarian remnant *in situ*, was grafted into the spleen, the entire ovary being unable to afford any protection through production of steroids. Nevertheless mammary, vaginal and uterine changes characteristic of ovarian fragmentation failed to appear (Niedmann, 1947; Bruzzone, Lipschutz and Niedmann, *unpubl.*). In similar experiments the steroid control is exerted by the ovarian remnant only, whereas the intrasplenic graft can act only by consumption. Protection was indeed not so complete as when the ovarian remnant was accompanied by the second ovary *in situ*; hemorrhagic follicles occurred though only exceptionally. That consumption of gonadotrophins influences in some way the anterior lobe has been shown by the work of Jungck et al. (1947): the hypophysis of a rat with both ovaries grafted into the spleen does not elicit in the infantile rat the same great increase of ovarian weight as the hypophysis of castrated rats does.

I should like to lay stress on the fact that the above tentative explanation of the sequels of ovarian fragmentation does not hold for the overthrow of the ovarian-hypophysial balance which is effected by so many other operative interferences on the ovary as transplantation, traumatization by incisions, vasodilatation by painting the blood vessels with phenol, or ligature of ovarian blood vessels, to which reference has already been made (p. 191–192). There is in all these experimental conditions which seemingly differ so widely one from another, the same *lasting* ovarian-hypophysial imbalance as with ovarian fragmentation, though the interference itself may be of short duration as with painting or ligature, or as with transplantation when the intrarenal graft in less than a fortnight begins again producing estrogen and releasing it into the general circulation. Thus it would seem that the hypophysial change which is effected momentarily by all these experimental procedures is *irreversibly maintained*. The question of the irreversibility of these hypophysial changes, or the question of the irreversibility of the imbalance, deserves greatest interest from the point of view of tumorogenesis in the body. It has been discussed in greater details but no definite conclusion could be reached (Lipschutz, 1938, p. 21–24)—and as to this we are to-day not more advanced than we were 12 years ago!

Neither must the concept of the supposed control by a balance between production and consumption be oversimplified. Chorionic gonadotrophins from the urine of pregnant women when introduced into the blood of an

experimental animal rapidly appear in the urine of the latter (Parkes and White, 1933; Hill et al., 1934; Evans et al., 1933). As much as 80 to 90 per cent disappears from the blood of the rabbit as early as 10 hours after intra-venous injection (Lipschutz and Vivaldi, 1934; Lipschutz, Fuente-Alba and Vivaldi, 1935a). But injected chorionic gonadotrophin diminishes in the blood, though less rapidly, even when the rabbit has been previously nephrectomized (Lipschutz, Fuente-Alba and Vivaldi, 1935b). The mare gonadotropic hormone also disappears from the blood of the rabbit, intact or castrated; it disappears less rapidly than chorionic gonadotrophin and without being excreted with the urine or feces (Catchpole et al., 1935). It was remarkable that in our work with nephrectomized rabbits only about 30 per cent disappeared in the course of 10 hours instead of 80 per cent in intact animals, and that the figure for chorionic gonadotrophins in nephrectomized animals was coincident with that for mare gonadotrophins in animals with intact kidneys, castrated or not. Neither is there a storage in the uterus, spleen, lungs, kidneys or liver (Catchpole et al., 1935). The above data show conclusively that gonadotrophins may disappear from the body in various ways, not only by consumption in the gonads or excretion with the urine. The metabolism of the gonadotrophins is a problem which has to be met with. The only fact known is as yet a negative one, that is to say, that gonadotrophins are not inactivated in the liver when injected into a mesenteric vein of the female rabbit; this refers both to chorionic and mare serum gonadotrophins (Hamburger and Pedersen-Bjergaard, 1946b).

2. Abnormal tubular growths in the ovarian hilus, and their virilizing action

In female guinea pigs with ovarian fragmentation masculinization may occur; the clitoris may grow and take the aspect of a small hypospadiac penis (fig. 107). The condition is similar to that known from an observation I made about thirty five years ago in a female guinea pig of Steinach's, and from the findings of Sand in female rats, with testicular grafts. The same condition may be found occasionally in females otherwise normal (Lipschutz, 1927a). This masculine transformation can be obtained in the guinea pig in a couple of days with the administration of testosterone propionate and other androgens (see table 12). It seems likely that an ovarian remnant may produce, though rarely, some androgen, or that it may stimulate its production in the adrenals. In most of our animals no histological structure in the ovarian remnant was found which might have been related to production of androgen, with the exception of two cases with great development of tubular structures probably belonging to the ovarian hilus (fig. 108) (Lipschutz, 1938). The tubules were lined with cells rich in protoplasm. Since tubular structures with so well developed an epithelial

lining were absent in the ovarian remnants of the remaining masculinized animals it seems not very likely that they were the producers of androgen.

In many of our ovarian remnants, especially in experiments of long duration, up to 33 months, large cysts were found. We took them for follicular cysts; but in a reexamination of these fragments we reached the conclusion that in any case most of these structures are cysts of the rete. All these older findings acquire now greater interest because it has been reported more recently that atypical growth of tubular structures of the ovarian hilus may take place under very different experimental conditions whose

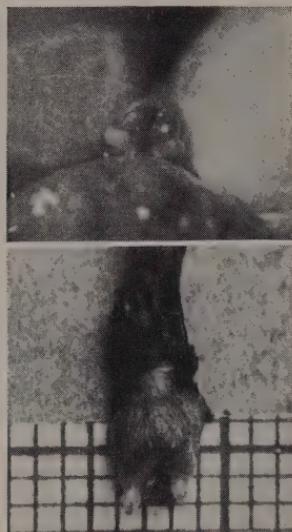


FIG. 107. Masculinizing action of ovarian remnant in g. pig. 38 days after operation. $\times 3.5$. (Ch.1205). A. The corpora cavernosa of the clitoris have been transformed into a penis-like organ. Laterally—horny styles characteristic of the penis. B. The penis-like organ excised after necropsy. The ovarian remnant contained an hemorrhagic luteic cyst; the vaginal mucosa was of the pregnancy type.

common denominator is the hormonal imbalance. Structures thought to be of rete origin have been found by Pfeiffer (1939) in female mice in which a hormonal imbalance was established by grafting testicles soon after birth. These cysts occupied sometimes a great part of the ovary. They were limited to one strain (C57). Raynaud (1941; many references) reported abnormal tubular structures in mice receiving estrogen. Tubular "adenomas" have been described in the ovaries of old mice (Engle, 1943), and similarly in various mammals receiving estrogen (Champy, 1937; Mosinger, 1949).

Abnormal tubular structures are not uncommon in intrasplenic ovarian

grafts. They may attain exceptionally a nodular aspect (fig. 109). Their origin from the rete is here quite evident; whether they originate by pro-

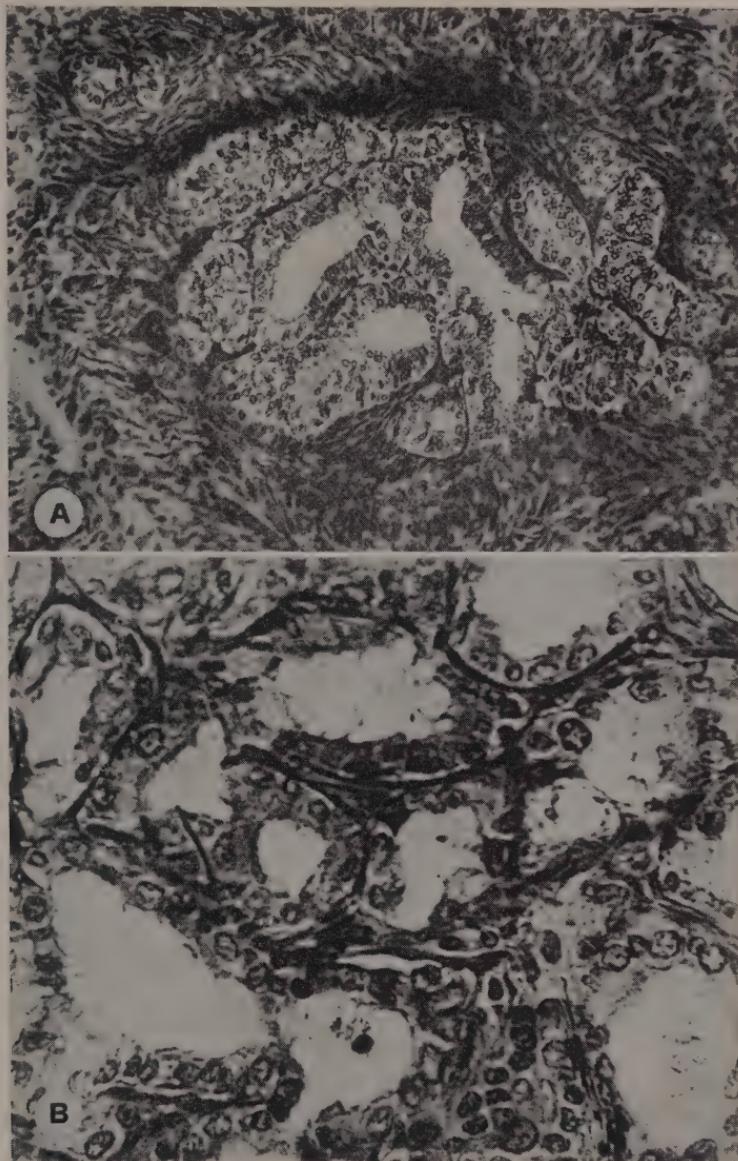


FIG. 108. Tubular structures in the hilus of ovarian remnant of g. pig, $5\frac{1}{2}$ mo. after operation. A. Tubules surrounded by connective tissue, probably hypertrophic medullary tubules. (Ch.1187). $\times 180$. B. Similar experiment. (Ch.1110). $\times 460$.

liferation or only by transformation of existing cells cannot be said with certainty. These tubules seem capable of migration; they may be found also outside the ovary in the splenic parenchyma, and on the surface of the

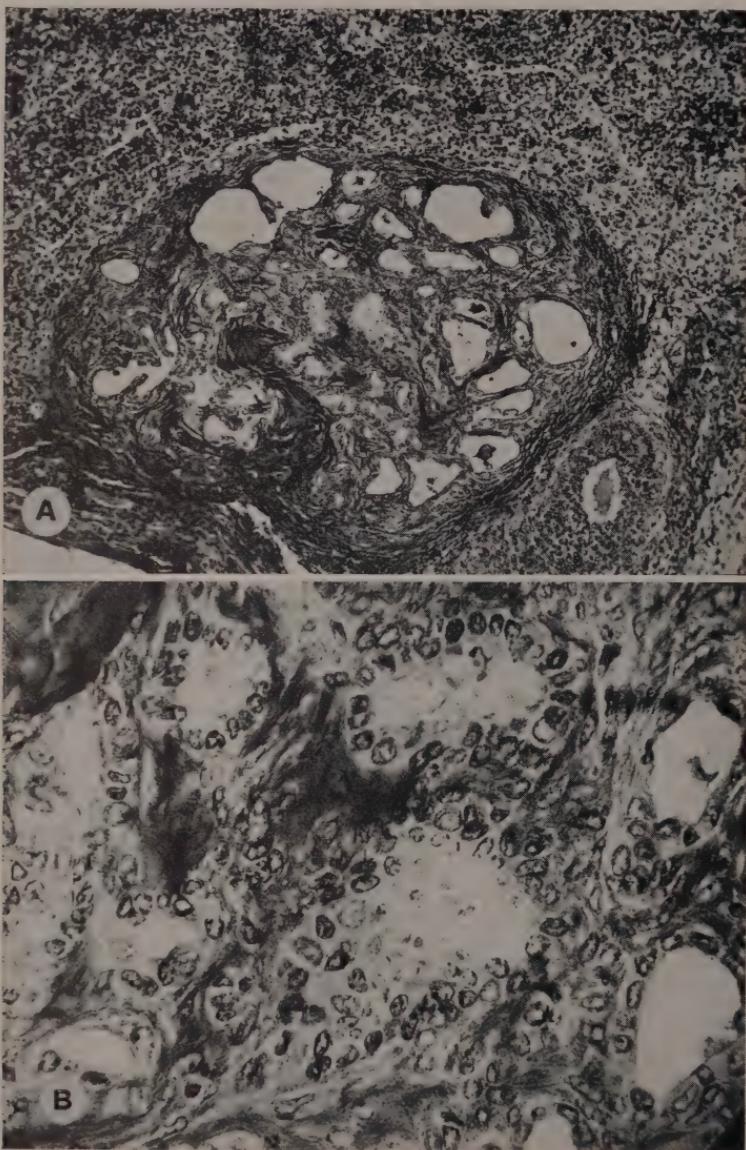


FIG. 109. Intrasplenic ovarian graft in castrated female g. pig, 11 months after grafting. Nodule protruding from the ovarian border into the spleen. A. $\times 90$. B. $\times 400$. (CXXVI.114).

spleen (Lipschutz and Iglesias, *unpubl.*). As there is, with intrasplenic ovarian grafts in castrates, an increased gonadotrophic activity of the hypophysis one may suggest that most of the reported atypical tubular structures of the rete are related to an ovarian-hypophysial hormonal imbalance though produced in different ways.

We shall refer now to masculinization which has been found, though only once, in a castrated male guinea pig with an ovarian graft; this exceptional condition also has been found to be concomitant with atypical growth in the ovarian hilus (Lipschutz, 1932, 1938). The ovary had been grafted about 3 years ago into the kidney. There was development of the

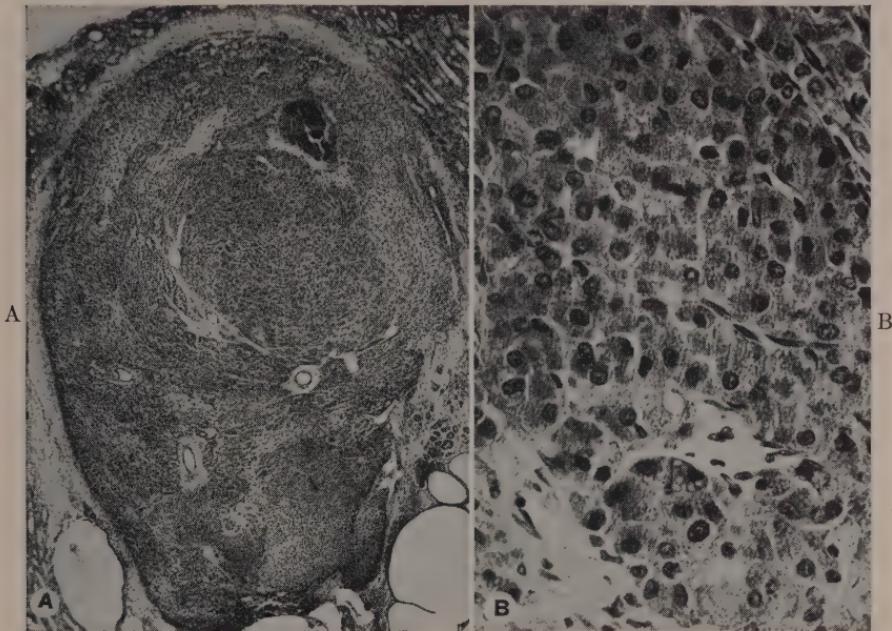


FIG. 110A AND B

nipples and mammary glands; but the penis, seminal vesicles and prostate behaved as in a normal male. The graft consisted of luteal cells very similar to those of a fully developed corpus luteum, disposed mostly in forms of solid cords (fig. 110A and B). But there were also tubular structures (fig. 110C), most probably derived from the hilus of the ovary. At one place the picture was highly suggestive of tubular structures undergoing atypical nodular proliferation (fig. 110D). Whereas it was fully evident that this graft behaved, both structurally and functionally, essentially different from intrarenal ovarian grafts in general we are unable to say which part of the graft was producing the androgen. Neither would it be wise to utter about

how far this exceptional structure in the guinea pig has to be related to what has been called in human pathology "arrhenoblastoma" and by many

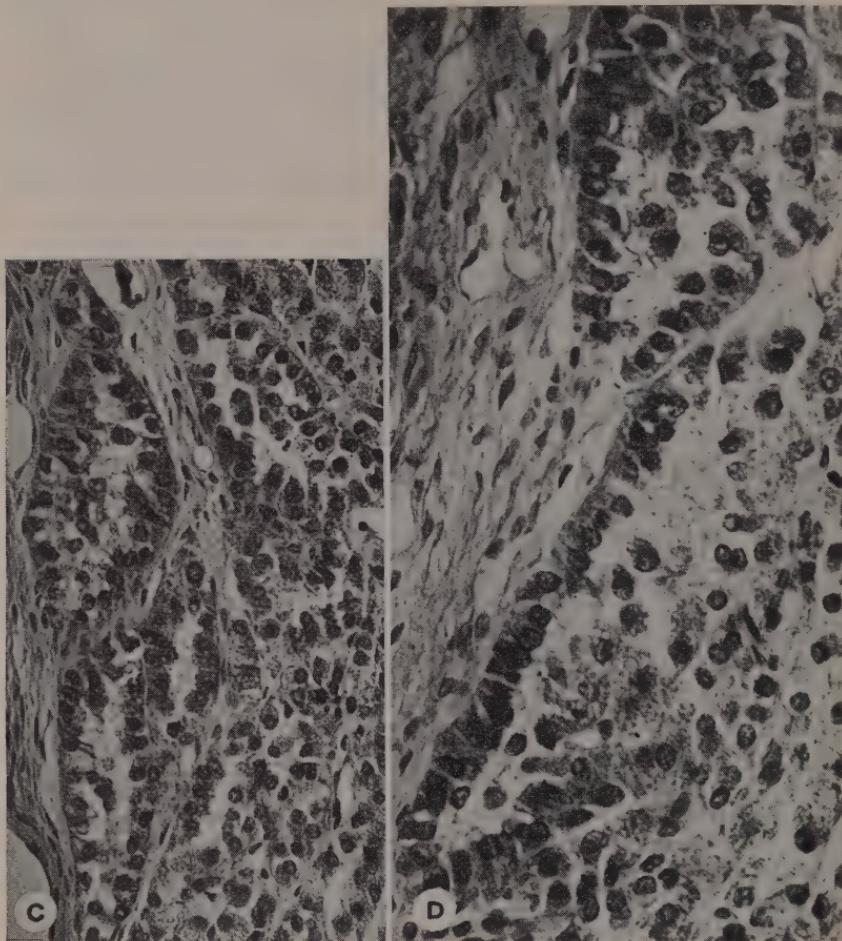


FIG. 110C AND D

FIG. 110. Masculinizing intrarenal ovarian graft in male g. pig, 34 mo. after grafting. (Ch. 319). A. Graft separated from renal tissue by collagenous capsule. Somewhat to the right from the center—large formation deriving probably from a corp. lut. To the left of latter—tubules. Almost the whole graft consists of epithelial cords. $\times 33$. B. Cords and clusters of epithelial cells. $\times 255$. C. Tubules; probably of medullary origin. $\times 250$. D. Two tubules, partly with atypical proliferation of the wall. $\times 250$.

other names (see Selye, 1946, p. 75; Willis, 1948, p. 500). It might have been due to a spontaneous tumoral growth already present in the ovary before. But in the light of modern knowledge on experimental ovarian

tumors originating in intrasplenic ovarian grafts it would seem equally justified—in any case more stimulating!—to assume that the described atypical growth was due to an experimentally established ovarian-hypophysial imbalance.

Whereas masculinization by an ovarian graft is so exceptional in *guinea pigs* it is frequent in the *rat* as has been discovered by Hill. The masculinizing effect was not correlated in the rat with any special histological structure of the graft (Hill, 1937; 1941; Deanesly, 1938). This is coincident with our findings in otherwise normal female guinea pigs in which a small hypospadic penis was found (Lipschutz, 1927a) though no abnormal microscopical ovarian structure was present. Masculinization of the genital region has been produced in guinea pigs also with X-rays or with the administration of hypophysial or chorionic extracts by which excessive ovarian luteinization was induced (Steinach and Kun, 1931; Papanicolaou and Falk, 1934; see later literature in Parkes, 1945). Consequently spontaneous masculinization in guinea pigs also has been attributed to excessive luteinization occurring at advanced age (Guyénot, Ponse and Wiltzkykowska, 1932). But later on the same group of workers has shown that masculinization can be induced in the female guinea pig with the administration of hypophysial and chorionic extracts in absence of the ovaries (Guyénot and Naville-Trolliet, 1936). This corroborates the former statement that spontaneous masculinization may occur also in young females and that it persists after removal of the ovaries (Lipschutz, 1927a); it has also been found that masculinization in the guinea pig as induced with X-rays is not concomitant with an ovarian histology different from that in non masculinized females (Genther, 1934). It is very likely that in all these cases androgens were in play given up by the adrenals. X-raying of the ovaries might have stimulated the corticotrophic activity of the anterior lobe.

3. Neoplastic growth in intrasplenic ovarian grafts

Experiments with intrasplenic ovarian grafts have opened a new field of research on ovarian tumors. The condition of the graft, and especially the appearance of blood follicles as observed in the guinea pig, is indicative of a gonadotrophic hyperactivity of the hypophysis. There is an overthrow of gonadal-hypophysial relationship due to a failure of the steroid control, and a consecutive increase of production, or release, of gonadotrophins.

We have already referred to the enormous increase of the ovary grafted into the spleen in the rat and guinea pig (fig. 86). The dynamics of this ovarian increase and its significance have been studied especially in the latter species where things develop more slowly than in the rat or mouse. At the beginning, till about 2 months after transplantation, the increase is due in the guinea pig almost entirely to blood follicles; later on corpora

lutea prevail (figs. 87 and 88). But there is from the beginning also another very remarkable ovarian pattern different from the normal one—the appearance of clusters of luteinized cells in the stroma, and their conflux. In time, at about 10 months, luteinization becomes highly pronounced; the irregular clusters of luteinized cells are sometimes ubiquitous (fig. 111). The disorderly luteinization of the stroma may set in long before corpora lutea become the prevailing structure of the graft. There were also signs of disorderly luteinization of the granulosa. Later on the cells of the corpus luteum may become intermingled with cells of the stroma showing a considerable variation in shape, size and chromatin structure of the nucleus. One cannot restrain from referring to the disorderly luteinization as to tumoral growth, (Lipschutz, Ponce de León et al., 1946). The picture is probably different as to its details from any ovarian tumor in the woman; but there is certainly a resemblance with what has been described as "lutein cell tumor" deriving from a granulosa cell tumor when undergoing ample luteinization (Geist, 1942; compare our fig. 111A and B to Geist's fig. 164, 166, and 185).

The Biskinds were the first to describe tumoral growth in the intrasplenic ovarian graft (M. S. Biskind and Biskind, 1944; G. R. Biskind and Biskind, 1949). They have interpreted the structure produced in the *rat*, 11 months after transplantation, as a non-luteinized granulosa cell tumor sharply demarcated from the masses of luteinized cells (see also G. R. Biskind, Pencharz and Biskind, 1948).

Tumors have been reported also in intrasplenic grafts in *mice* (Li and Gardner, 1947a; Li, 1948; Furth and Sobel, 1947b). This species has been shown to be the most propitious for similar studies; the findings made in this field in mice, together with work accomplished with x-rays in the same species, are a most outstanding recent progress in experimental tumorigenesis. Granulosa cell tumors, luteomas and mixed tumors were produced in intrasplenic grafts both in castrated females and males, mostly in the course of no less than 6 to 7 months; granulosa cell tumors have been reported also in intrapancreatic grafts in castrated mice (Li and Gardner, 1947b). The tumors derive apparently from ingrowing germinal epithelium (Li and Gardner, 1947c); the incidence is considerable and may reach as much as 67 per cent (Furth and Sobel, 1947b). Metastases in the liver may occur (Li, 1948). The tumors are transplantable beneath the skin (Li, Pfeiffer and Gardner, 1947; Li, 1948). But subcutaneous and intraperitoneal transplants may also fail, and intrasplenic passages may be necessary so as to obtain transplantability; successful transplants may produce metastases in the liver and lung (Furth and Sobel, 1947b). There was no strain limitation as to these tumors in mice (Li, Gardner and Kaplan, 1947).

Neoplastic degeneration has been reported also in *testicular* intrasplenic grafts in *rats* at 11 months after transplantation; the tumor resembled closely to the granulosa cell tumor in ovarian grafts (Biskind and Biskind,

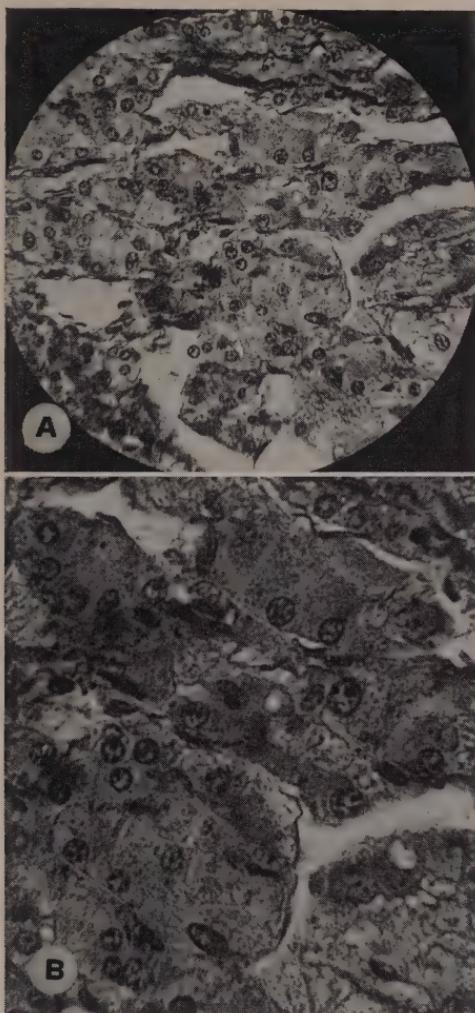


FIG. 111. Luteomatous condition of ovarian stroma in intrasplenic grafts in g. pigs. Same ovary as fig. 88D. 303 days after grafting. A. Cords of luteinized cells. $\times 200$. B. Same. $\times 400$.

1945). Other workers describe interstitial cell tumors in testicular grafts in the rat (Twombly, Meisel and Stout, 1949). But testicular tumors did not arise in *mice* in the course of 9 months (Li, Pfeiffer and Gardner, 1947), or

even when the experiment lasted as long as 12 months (Vivien, 1948a). Hyperplasia of interstitial tissue took place in the testicle grafted into the hilus of the spleen in the *guinea pig* in the course of 3 months; but we are unable to say whether this growth was more pronounced than in retained testicles in general (Iglesias and López, unpubl.).

As has been demonstrated especially in the guinea pig the pattern of the intrasplenic ovarian graft undergoes considerable changes with the time (figs. 87 and 88). Is this time-conditioned ovarian change, say from 2 to 10 months, due to a change of ovarian reactivity, or to a time-conditioned functional change of the hypophysial gonadotrophic activity? One may tentatively assume that there is an interplay of both these factors. But whichever the details may be there is the definite fact that tumoral growth of ovarian tissues can be elicited through a failure of the ovarian control of the hypophysis having lasted for a sufficient length of time. As evidenced by work with mice related above chemical stimuli, as hypophysial gonadotrophins, though normal as to quality, but acting in increased or altered relative quantities and in uncontrolled timing conditions, are able to induce hitherto normal ovarian cells to deviate from their normal developmental path and finally also from their normal reactivity and to acquire neoplastic characters as exemplified by autonomous growth and metastases. There can be no better example of the intricate relationship existing between normal and atypical growth, and between benign and malignant tumoral growth, a relationship on which stress has been laid in chapter 9 of the Part I.

These ovarian tumors exemplify at the same time how a failure of the steroid control of hypophysial function may be at the root of malignant neoplastic growth. The latter has been further substantiated by the fact that abnormal growth never occurs in the intrasplenic ovarian graft when the second ovary is left in the body *in situ* (p. 178). Even more convincing are experiments with intrasplenic ovarian grafts and the simultaneous administration of steroids. Pellets of progesterone were implanted into guinea pigs in which an intrasplenic graft was made 9 months ago the second ovary having been removed. About 120 to 175 µg of progesterone per day were absorbed during 3 months; not a single animal with the luteomatous transformation of the stroma was discovered. Most corpora lutea were in a state of degeneration (Mardones, 1948; Iglesias, Lipschutz and Mardones, 1948, 1950a). On the contrary, as much as 1 mg of progesterone per week administered by injection did not inhibit production of ovarian tumors in mice; inhibition took place with estradiol benzoate and testosterone propionate (Li, 1948; Li and Gardner, 1949). The difference in the response of guinea pigs and mice to progesterone might have been due simply to the fact that continuous absorption from a subcutaneous pellet was more effec-

tive than discontinuous absorption of the hormone given by injections. Progesterone was apparently able to prevent the growth of a transplanted granulosa cell tumor which had been originally induced by intrasplenic transplantation in mice (Clifton and Pan, 1948). But a differential response to progesterone of the two species also has to be considered.

4. Atypical ovarian growth induced with X-rays

The authorities who have worked with intrasplenic grafts in rats and mice have attracted attention to the structural similarity between the tumors originating in these grafted ovaries and those readily induced in mice by X-rays. Transformation of the whole ovary, through irradiation, into interstitial tissue of a glandular aspect with an enhanced secretion of female hormone has been described in guinea pigs as early as 34 years ago; there was considerable growth of the nipples, of the mammary glands which may secrete milk, and of the uterus (Steinach and Holzknecht, 1916); that is to say, there was an increased and continuous action of estrogenic hormone, according to modern knowledge. It has been concluded from these and other experiments that under the influence of X-rays endocrine cells are, so to speak, poured into the stroma in increased number thanks to enhancement of follicular atresia (Lipschutz, 1924, p. 253). Subsequently full corroboration has been given to this tentative explanation. It has been proved that the hypertrophied interstitial cells which constitute the irradiated ovary derive from follicles undergoing atresia (Genther, 1931), not only in the guinea pig but also in mice (Parkes, 1926-1927; Brambell, Parkes and Fielding, 1927-1928) and rats (Levine and Witschi, 1933).

Ovarian neoplasms have been produced in the remarkable work of Furth and his students by X-rays in mice (Furth and Butterworth, 1936; Furth and Furth, 1936; Butterworth, 1937; also Geist et al., 1939, 1941). The tumors arose 7 to 10 months after irradiation. It has been assumed that tumors failed to appear in the work of Parkes and Brambell et al. because these authorities terminated their experiments at about 6 months after irradiation (Furth and Boon, 1947); there is seemingly no sharp limit between X-ray induced hyperplasia of the interstitial tissue and neoplastic growth (Butterworth, 1937).

These ovarian growths have been classified by Bali and Furth (1949) as (1) tubular adenoma deriving from the germinal epithelium, (2) granulosa cell tumors possibly deriving from the former, (3) luteomas deriving from granulosa cell tumors, (4) sarcomas, angiomas and endotheliomas. The tumors are malignant: they are readily transplantable, and this refers both to the granulosa tumor (Furth 1946; Furth and Boon, 1947) and the luteoma (Furth and Sobel, 1947a; Bali and Furth, 1949). Their transplantability is apparently much superior to that of the tumor of similar

structure originating in the intrasplenic graft, and for this reason the latter has been interpreted as not yet fully autonomous when compared to tumors induced by X-rays (Furth and Boon, 1947). Many of these tumors show invasiveness; metastases to lung, kidney and liver have been described (Bali and Furth, 1949).

There can be scarcely any doubt that these tumors are due, like the atypical growth in ovarian fragmentation, or the tumoral growth in intrasplenic grafts, to an overthrow of the ovarian-hypophysial relationship. As already referred to there was in the early work which X-ray irradiation in guinea-pigs a severe disturbance of the ovarian endocrine function (Steinach and Holzknecht, 1916; Genther, 1931) indicative of a considerable lengthening of estrogenic action; the same result has been reported with rats (Levine and Witschi, 1933). Cystic glandular hyperplasia of the endometrium and, in one case, a uterine myoma has been reported in the guinea pig (Schmidt, 1939). It is true there were regular estrous cycles in the work of Parkes, Brambell and Fielding (1926-1928), though the structural condition, especially in animals exposed to X-rays when already adult, was seemingly coincident with that known from the older work in guinea pigs. But there was also in mice an unusual feature in the post-irradiation cycle similar to that observed in a more pronounced manner in the irradiated guinea pig: the slightly greater variability in the length of the cycle (Parkes, 1927, Part III; 1929, p. 144). Later on a considerable disturbance of the sex cycle may occur; so great are the irregularities that estrous may last as long as 63 days, or anestrous may last as long as 222 days (Geist et al., 1939). The overthrow of the ovarian-hypophysial balance is due primarily to the change induced in the ovary; this is shown by the fact that no ovarian abnormality is produced when the pituitary region is irradiated and the ovarian region is shielded, whereas the characteristic ovarian changes occur when the ovarian region is irradiated and the pituitary region is shielded (Furth and Boon, 1947). "The exposure to X-rays may last only for seconds but the chain of events which follows covers the entire life span of the animal" (Bali and Furth, 1949). These are impressive words, and no less impressive facts!

One may suppose that, similarly to what occurs in ovarian fragmentation or in other ovarian lesions, the irradiated ovary loses the faculty to control the hypophysis the latter undergoing subsequently an irreversible change (p. 220). Consequently—and again similarly to what occurs in ovarian fragmentation—an intact ovary *in situ* protects against any remarkable hypophysial change, as shown by various workers. When in guinea pigs one ovary was shielded and the second one was irradiated, the latter did not show the hyperplastic condition though most of the follicles had undergone degeneration; there was no disturbance of the sexual cycles

(Schmidt, 1938). The non-irradiated ovaries contained, in compliance with the law of follicular constancy, an average of 2.3 corpora lutea (Schmidt, 1938), that is to say, not much less than the two normal ovaries together the average size of a litter being of 2.7 (Lipschutz and Adamberg, 1925). The law of follicular constancy was complied with also in mice with only one ovary irradiated though the latter showed the same irradiation changes as with both ovaries irradiated (Brambell and Parkes, 1929). But no tumors were induced in similar irradiated ovaries the second ovary having been left intact, even when the experiments lasted 16 months (Lick et al. 1949).

Stress has been laid on the fact that the behavior of the intrasplenic graft varies according to the species (p. 227); it suffices to compare results with guinea pigs and mice. It is the same with the response of the ovary to X-ray treatment. No ovarian tumors have been reported in the work with irradiated guinea pigs though deep going ovarian changes were produced (see above). A difference even more remarkable becomes evident when rodents are compared to Rhesus. As already mentioned subtotal removal of the ovaries failed to produce in this species any changes in the ovary or the genital tract (p. 192). Neither were changes found in the ovaries or the endometrium when Rhesus was repeatedly exposed to X-rays (Dahl-Iversen and Hamburger, 1947). Since cystic glandular hyperplasia of the endometrium and cervical metaplasia can readily be elicited in Rhesus with the prolonged administration of estrogen (references Zuckerman, 1940; Dahl-Iversen et al., 1942; Engle et al., 1943), one must assume that resistance against X-rays in Rhesus is due to a special genetically fixed functional pattern of the ovarian-hypophysial complex. Incidence or non-incidence of certain types of neoplasms in the ovary and genital tract may thus be expressed in terms of a functional condition intimately related to the steroid balance in the body.

The results which have been exposed in this section are very likely to strengthen the concept that granulosa cell tumors, luteomas and related neoplasms in women are but the offspring of originally normal constituents of the ovary. But these constituents have undergone an abnormal developmental process, due to the interference of exogenous or endogenous stimuli not foreseen—as to quantity and timing—in the normal course of evolutionary events. The idea that these neoplasms spring up from some ovarian structure abnormal “ab ovo” held by so distinguished pathologists as Meyer and Novak (see Novak, 1941) has to give now definitely the way to the new concept inspired by experimental research (see especially Furth and Sobel, 1947a; Bali and Furth, 1949; Willis, 1948, p. 496; also Geist and Spielman, 1943; Selye, 1946, p. 51–73.)

Admitting that a hormonal imbalance is at the root of ovarian tumors

in X-rayed animals Furth postulates that a delayed chromosomal change induced by X-rays in the ovary and coupled with the hormonal imbalance also might be in play. This concept is based on the fact that mice X-rayed at one to three days of age, or at a few weeks of age, can go through several pregnancies although no less than 76 per cent of these mice are destined to develop ovarian tumors at 8 to 23 months (Furth, 1949; Bali and Furth, 1949). The fact that functional changes become manifest only after such a considerable latent period is, indeed, in itself of fundamental interest in tumorigenesis. It has been reported that structural changes can be observed in the ovary as early as one hour after irradiation (Van-Eck-Vernande and Freud, 1949). But whether a delayed chromosomal change is the true explanation has still to be studied in greater detail. The question of initiation and promotion is with ovarian tumors in X-rayed animals, or in intrasplenic grafts, as patent as in experiments with carcinogenic hydrocarbons or estrogens (see table 6, p. 116).

C. NEOPLASTIC GROWTH OF THE ADRENAL CORTEX

The adrenal cortex is dependent on ovarian hormones (summary Parkes, 1945). Interest has been attracted to the adrenals in the field of tumorigenesis related to steroids already years ago when estrogen-induced changes in the X-zone, or the "brown degeneration" between medulla and cortex (Burrows, 1936c), were reported in a genetically pure strain of mice with a high incidence of spontaneous mammary cancer (Cramer, 1937); they were shown to set in before the onset of the latter. However when 6 inbred strains of mice with a high incidence of mammary cancer and 5 strains free, or almost free, of mammary cancer were studied comparatively, no consistent relation between frequency of brown degeneration and the mammary tumor was found (Blaisdell, Gardner and Strong, 1941; with literature on "brown degeneration").

On the other hand, it has been found that under experimental conditions by which a gonadal-hypophysial imbalance is produced tumors of the adrenal cortex may originate. This discovery was due to the fundamental work of the Little and Woolley group. Reproduction, or its opposite, i.e. castration performed in females of the *dba* strain, did not affect the incidence of mammary cancer in this strain in which about 50 per cent of virgin animals develop the breast tumor; there was an other unexpected finding in these castrated mice—a nodular hyperplasia of the zona granulosa of the adrenal cortex (Fekete, Woolley and Little, 1941). This nodular hyperplasia appeared also in castrated males (Woolley, Fekete and Little, 1941). It was soon recognized that in other strains the adrenal response proceeds, under the same experimental conditions, much farther than that adrenal carcinoma originating in animals castrated at birth; and that a hormonal

imbalance is at the root of this tumoral response (Woolley, Fekete and Little, 1943). In females of the *ce* strain castrated 1 to 3 days after birth the incidence of adrenal carcinoma may attain at 6 to 12 months as much as 100 per cent (Woolley and Little, 1945a). The tumor was bilateral in 69 per cent of the females, and in 46 per cent of the males (Fekete and Little, 1945b). Both in females and males, estrogen is produced by the adrenal tumor; at a given moment signs of castration begin to disappear, the vagina opens, the mammary glands and the uterus develop; there may be cystic glandular hyperplasia and mammary cancer. But the tumor produces also androgen; enlargement of the clitoris and masculinization of the submaxillary gland take place (Woolley and Little, 1945a). Metastases are produced (Woolley and Little, 1945a); the tumor is transplantable (Woolley and Little, 1946). Adrenal carcinoma has been elicited in females castrated at the age of 43 to 65 days also in the NH strain of the Yale group, and again with an incidence as high as 13 out of 15 animals (Gardner, 1941c); the estrogen in the urine and feces which were collected for many months, was 4 times that in normal females (Dorfman and Gardner, 1944).

One must assume that these results are due to a gonadal-hypophysial imbalance (Woolley et al., 1943), that is to say, that the *gonadal* control of the anterior lobe is fundamental also for the *corticotropic* hypophysial function and not only for the gonadotrophic one; and secondly, that an alteration of the gonadal-hypophysial relationship may lead to tumoral growth including cancer in other organs than the gonads. It has been found recently that in gonadectomized mice with adrenal tumors basophilic *hypophysial tumors* also may appear subsequently (Dickie and Woolley, 1949). The hypophysial tumor was secondary to the adrenal changes. But according to the authorities fundamental changes might have occurred first in the pituitary which reacted on the adrenals.* The concept of a hypophysial impairment underlying adrenal changes is substantiated also by the statement that in mice with intrasplenic grafts, that is to say with an ovarian-hypophysial imbalance, the adrenals may contain nodules similar to those described by the Little-Woolley group (Furth and Sobel, 1947b). It was remarkable that these adrenal nodules appeared only when the intrasplenic graft did not transform into an ovarian neoplasm; one may tentatively assume that with a given genetically fixed reactivity of the hypophysis various patterns of hypophysial impairment may originate under variable experimental conditions and that these patterns manifest

* The implication of corticotrophic hormones is warranted by an observation of Flaks (1949) though, indeed, referring only to a single animal. A Strong A mouse ovariectomized at 3 days and receiving adrenocorticotrophic hormone over a period of 8½ months was autopsied at an age of 537 days. A highly malignant cortical carcinoma was found; the tumor invaded the kidneys and stomach; there were metastases in the liver.

themselves in tumoral responses of different organs as the adrenals, the gonads, the mammary glands and the anterior hypophysis itself. These and other findings also strongly suggest that the tumor itself interferes in the hypophysial hormonal activities. Transplants of cortical carcinoma exert a restraining action on the occurrence of primary cortical tumors (Woolley and Little, 1946b). As already mentioned the cortical tumor produces gonadal hormones (Woolley and Little, 1945a; Frantz and Kirschbaum, 1949a). But so far there is no certainty as to whether the tumorigenic faculty of the anterior lobe can be controlled by physiological quantities of gonadal hormones. Whereas the appearance of cortical carcinoma was prevented by convenient amounts of stilbestrol (Woolley and Little, 1946a) this was not the case when estrogen was administered in quantities sufficient to maintain continuous estrous (Frantz and Kirschbaum, 1949b).

Spontaneous adrenal cortical tumors also occur among mice. Dalton et al. (1943) reported a tumor without metastases; but the growth was transplantable. More recently it has been discovered that in the NH strain of mice adenomas of the adrenal cortex appear spontaneously in more than 90 per cent of females of over 1 year of age and, though rarely, also in males; in one case an histologically malignant cortical tumor was found in a male at the age of 23 months (Kirschbaum et al., 1946).

Adrenal *medullary* tumors have been reported in castrated or irradiated mice of different strains; they were thought to be small pheochromocytomas (Smith et al., 1949).

Chapter 22

TABULATED SUMMARIES OF THE THIRD PART

A. HYPERPLASTIC, METAPLASTIC AND NEOPLASTIC CONDITIONS DUE TO THE ACTION OF ENDOGENOUS HORMONES, UNDER AN EXPERIMENTALLY INDUCED GONADAL-HYPOPHYSIAL IMBALANCE

Experimental procedure	Pathological condition	Species	Author
I. Ovarian fragmentation	1. Cystic glandular hyperplasia of the endometrium	Guinea pig	Burch, Wolfe et al. (1932), Lipschutz (1936, 1937)
	2. Adenomatous polyps of the endometrium		
	3. Insular stratified metaplasia of the endometrium		
	4. Squamous metaplasia of uterine cervix, with cornification		Lipschutz (1936, 1937b, 1938)
	5. Epithelioma of uterine cervix		
	6. Invasion of proliferated uterine glands into the myometrium		
	7. Uterine fibromyoma		Morató (1941), Nadel (1949)
	8. Extrageneral fibromyoma		Nadel (1949)
	9. Follicular cysts of the ovary		Lipschutz (1928)
	10. Luteic cysts, sometimes hemorrhagic		Haterius (1930)
	11. Cysts of the rete of the ovary*		Lipschutz (1931, 1937b, 1938)
	12. "Adenomatous" nodules of the rete of the ovary		Lipschutz & Voss (1925)
II. Traumatization of the ovary by incisions	13. Follicular cysts	Rat	Wang & Guttmacher (1927)
III. Painting of ovarian blood vessels	14. Follicular cysts	Rat	Haterius (1930)
IV. Ligature of ovarian blood vessels	15. Cystic glandular hyperplasia of endometrium	Rat	Fels (1938, 1942, 1944a)
	16. Follicular cysts		
V. Testicular fragmentation	17. Nodules of the interstitial cells	Guinea pig	Lipschutz (1924)
VI. Testicular grafts in newborn females	18. Cystic glandular hyperplasia of the endometrium	Mouse	
	19. Adenomatous polyps of the endometrium		
	20. Invasion of proliferated uterine glands into the myometrium		Pfeiffer (1939)

* Formerly interpreted by us as "follicular cysts."

TABULATED SUMMARIES OF THE THIRD PART—(Contd.)'

Experimental procedure	Pathological condition	Species	Author
	21. Uterine myoma 22. Uterine adenocarcinoma; <i>metastases; invasion</i>	Mouse Mouse	Pfeiffer (1949a) Pfeiffer (1949b)
VII. <i>Intrasplenic ovarian graft</i>	23. Fibrous nodules on the surface of the spleen 24. Follicular cysts 25. Luteic cysts, sometimes hemorrhagic 26. Luteomatus evolution of the ovary 27. "Adenomatous" nodules of the rete of the ovary 28. Granulosa-cell tumor 29. Granulosa-cell tumor; <i>metastases, transplantable</i>	Guinea pig Guinea pig Guinea pig Guinea pig Rat Mouse, not strain-limited Mouse Mouse	Lipschutz (unpublished) Lipschutz et al. (1946) Iglesias, Lipschutz & Mardones (unpublished) M. S. Biskind & Biskind (1944) Li & Gardner (1947), Li et al. (1947), Li (1948) Furth & Sobel (1947b) Li & Gardner (1947) Furth & Sobel (1947b)
VIII. <i>Intrasplenic testis graft</i>	30. Luteoma 31. Adenomatous nodules of adrenal cortex 32. Testicular tumor (?)	Rat	M. S. Biskind & Biskind (1945), Twombly et al. (1949)
IX. <i>X-ray treatment of the ovary</i>	33. Hyperplasia of interstitial tissue deriving from follicles 34. Hyperplasia of interstitial tissue deriving from germinal epithelium 35. Granulosa-cell tumor; <i>metastases, transplantable</i> 36. Luteoma; <i>transplantable</i> 37. Tubular adenoma deriving from germinal epithelium 38. Sarcomas, angiomas, endotheliomas 39. Uterine myoma	Guinea pig Adult mouse Rat Infantile mouse Mouse Mouse Guinea pig	Steinach & Holzknecht (1916) Genther (1931) Parkes (1927) Levine and Witschi (1933) Parkes (1926, 1927), Brambell, Parkes & Fielding (1927, 1928) Furth & Butterworth (1936) Furth & Boon (1947) Furth & Sobel (1947a) Bali & Furth (1949) Schmidt (1939)
X. <i>Total removal of the ovaries</i>	40. Hyperplastic nodules of adrenal cortex 41. Carcinoma of adrenal cortex; <i>metastases, transplantable</i> 42. Mammary tumor in male 43. Cystic glandular hyperplasia of endometrium 44. Hypophysial tumor	Mouse Mouse, strain limited Mouse, strain limited Mouse	Fekete, Woolley & Little (1941) Woolley & Little (1945, 1946) Woolley et al. (1941) Gardner (1941) Woolley (1947), Dickie (1947)
XI. <i>Intrarenal ovarian graft</i>	45-48. Similar to 1 to 4 49. Follicular cysts of ovary† 50. Medullar growth of the ovary (masculinizing "arrhenoblastoma" or "tubular adenoma")	Guinea pig Guinea pig Guinea pig	Lipschutz (1937b, 1938) Lipschutz, Voss et al. (Lipschutz, 1927b) Lipschutz (1932, 1937b, 1938)

† Persistence of fully grown follicles, but only rarely of more than normal size.

B. ANTITUMORAL AUTODEFENSE, AND STEROID HOMEOSTASIS

Antitumoral Autodefense by

Experimental demonstration

I. Ovarian-hypophysial function:

- A. Rhythmic quantitative changes in the production of estrogens.
- B. Rhythmic production of progesterone.
- C. Steroid control of the gonadotrophic function of the hypophysis; intra-hypophysial (?) estrogen-progesterone antagonism.

1. Production of abdominal fibroids by the continuous action of estrogens.
2. No fibroids are produced when the action of estrogens is discontinuous.
3. Prevention of estrogen-induced fibroids with progesterone.
4. Prevention of fibroids also with the intermittent action of progesterone.
5. Luteinizing action of estrogen in experiments with intrasplenic ovarian grafts; progesterone antagonizes this action of estrogen.
6. Overthrow of the normal sequence of the follicular and luteal phase in an ovarian remnant (luteic cysts; hemorrhagic luteic cysts).
7. Atypical proliferation of interstitial tissue in a testicular remnant.
8. Atypical proliferation of ovarian tissues in the intrasplenic graft.

II. Suprarenal function:

- Production of 3-keto-steroids of antiestrogenic activity.

9. Prevention of fibroids with desoxycorticosterone and dehydrocorticosterone.
10. Antifibromatogenic quantities of desoxycorticosterone are not toxic.
11. Desoxycorticosterone antagonizes, like progesterone, the luteinizing action of estrogen in experiments with the intrasplenic ovarian graft.
12. Estrogen calls for the production of antiestrogenic substances (mucification of the vaginal mucosa with the prolonged action of estrogen in the castrated female).

III. Hepatic function:

- A. Inactivation of estrogens and other steroids.

13. No fibroids are produced when fibromatogenic quantities of α -estradiol are absorbed by the spleen.
14. Draining of the animal's own estrogens through the liver; inactivation persisted during 22 months.
15. Urinary estrogens (metahormones) are less fibromatogenic than ovarian estrogens.

B. ANTITUMORAL AUTODEFENSE, AND STEROID HOMEOSTASIS—(Cont'd)*Antitumoral Autodefense by**Experimental demonstration***B. Quantitative limitations of the inactivating faculty.**

16. No fibroids are produced when fibromatogenic quantities of urinary estrogens are absorbed by the spleen.
17. Fibroids are produced with intrahepatically implanted pellets of estrogens.
18. Estrogens produced in an intrahepatic ovarian graft reach the general circulation.
19. Certain artificial compounds (stilbestrol, hexestrol, benzoestrol) even when absorbed from intrasplenically implanted pellets may reach the general circulation and fibroids may be produced.
20. Estrous is established for several weeks with intrasplenic pellets in the castrated animal, before the phase of inactivation sets in.
21. Cystic glandular hyperplasia of the uterus may be produced with intrasplenic pellets before the phase of inactivation sets in.

C. Functional adjustments of the inactivating faculty.

Chapter 23.

CONCLUSION: SOME PRACTICAL ASPECTS

The object of this book was to offer to the research student and to the clinician a summary of *experimental* work. For this very reason it could not be our task to discuss details related to treatment of tumors by steroids in humans. These problems belong to the realm of the clinician, and being outside the immediate scope of our own research work it would have been presumptuous on our part to discuss them. But there are certain practical aspects of a more general character which I feel we are entitled to emphasize before closing this book.

We have seen that all estrogens without any exception, natural or artificial, free or esterified, can become toxic for the body, and tumorigenic. No serious observer or reader of clinical papers will doubt about this to-day. But to reject the use of estrogens for therapeutical purposes on account of atypical epithelial proliferation or even tumors which can be elicited in the breast or endometrium by the administration of estrogens, would be as antiscientific as rejecting the use of insulin on account of the fatal hypoglycemia so easily produced by this hormone. As with insulin, the problem of therapeutics with estrogens is that of the conditions under which this essential hormone acquires toxic action in the body, and how this toxic action can be avoided.

Under certain experimental conditions esterified estradiol is more toxic than the free hormone. But on the basis of our experimental knowledge it would be a real sophism to declare that esters of estradiol have to be avoided because they, and so also the artificial estrogens, are more active than the free natural hormone in eliciting atypical cellular proliferation as exemplified by experimental fibroids or epithelial proliferation in the genital tract. We must hold in mind that greater toxicity of the esters and of the artificial estrogens is due principally to more protracted absorption, and that the free hormone when continuously absorbed from a subcutaneously implanted pellet becomes as tumorigenic as are the most tumorigenic esters. When administered by implantation of pellets minute quantities of the free hormone are able to elicit fibroids and epithelial proliferations which may appear very early; when absorbed continuously these minute quantities of the free hormone are as tumorigenic as minute quantities of the most tumorigenic ester of estradiol, the 17-caprylate.

On the other hand, no tumors are induced even with considerable quantities of an highly fibromatogenic ester—the 3-benzoate—when the latter is administered in such a manner that there is only discontinuous action as in the sex rhythm. It derives clearly from our work that *the fundamental problem of therapeutics with estrogens is not searching for a "less toxic" or "less tumorogenic" compound but establishing those timing conditions of administration which guarantee discontinuous action of the estrogen.* Thus one of the essential problems of therapeutics with estrogens is *imitating the sexual rhythm.*

From this point of view it seems contrary to nature to administer estrogens uninterruptedly as is so often done by the practitioner. It seems likewise contrary to existing knowledge on the nature of estrogenic action in the body to administer estrogen by implanting pellets, or tablets, beneath the skin. From an experimental point of view a rational treatment with estrogen would be administering the hormone by mouth, for about two weeks, starting again after an interval of two weeks, and so on. Under these timing conditions of treatment *all* estrogens are harmless, as *all* estrogens are harmful when administered continuously, unless quantities so minute are given as to maintain the level of estrogen in the body always beneath the tumorigenic threshold concentration which indeed so far is *unknown* to us. And even this low concentration of estrogen in the body while *continuously* maintained, and not combined intermittently with the administration of progesterone, might well become harmful and tumorigenic. It would seem that any deviation from the rule of the rhythm is admissible only in the treatment of breast cancer in aged women, or in the treatment of prostatic carcinoma in men.

Quite different from these essential rules of therapeutics with estrogens are the rules which should underly the treatment with other steroids as androgens or progesterone when used with the special purpose of antagonizing the supposed action of tumorigenic substances. In the same way as the tumorigenic action of free estradiol increases fifty to hundred times when absorbed from a subcutaneously implanted pellet instead of being given by injections, so also the action of antitumorigenic steroids increases. In all attempts of hormonal treatment of tumors as uterine fibroids or mammary cancer preference must therefore be given to subcutaneously implanted pellets. It is certain that progesterone acts in the normal body rhythmically. When trying to imitate, especially in the castrated woman before the menopause, the sexual rhythm by a "bihormonic" treatment, that is combining estrogen and progesterone, administration of the latter must be rhythmical. But in treatment of tumors with antitumorigenic steroids the timing problem before us is fundamentally different from that in bihormonic treatment. One may tentatively suggest that full control of

continuity of action is essential also in the treatment of prostatic carcinoma with estrogens.

We are fully aware that it is not possible to apply to humans all those experimental results which have been obtained in laboratory animals. The behavior of homologous territories in different animal species towards tumorigenic estrogens, and so also towards steroids of antitumorigenic action as androgens and progesterone, varies enormously according to the species. It is to the practitioner, and in the first place to the gynecologist, to decide how far use can be made of the established experimental facts for the benefit of Man.

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* This paper has been unfortunately misshapen and many errors have crept in owing to an unforeseen stylistic intervention without the authors having read proofs.

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