STEROID HOMEOSTASIS HYPOPHYSIS AND TUMORIGENESIS

By

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From wide study and speaking of details
we come back to speaking of main lines.

Mencius, book VIII, ch. XV.
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FOREWORD

By Professor CHARLES HUGGINS, M.D.

Really it is not necessary to provide an introduction for this work or its distinguished author, but it is just to express publicly the veneration, the admiration and the affection which his contemporaries hold for Alexander Lipschutz and to commend this volume to a wide circle of readers who will surely benefit from its wisdom.

Well known to most of the communities of scholars of the world by his personal visits to receive their honours and to instruct, posterity will want to know more of this remarkable man than a scant biographical outline can convey. Alexander Lipschutz is a son of the Russian Baltic and an alumnus of the German Universities during one of their flourishing periods. His genius flowered early and at age 36, as Professor of Physiology at Dorpat, he founded the first of the Lipschutz schools. His second school was born after transplantation to the Universities of South America in 1926. It was in 1938 that the Government of Chile showed profound wisdom and established itself as a benefactor of science and of mankind by creating for Professor Lipschutz new laboratories in the Instituto de Medicina Experimental of the National Health Service in Santiago. From his famous Institute there have emerged a continous flow of important work and a distinguished school of young Chilean pupils who are well indoctrinated in the scientific method and are imbued with the highest ideals of cultivated man. The eminence of Chilean endocrinology was achieved through the effort of Alexander Lipschutz.

Alexander Lipschutz is a spare man of average height who conveys a Mosaic impression not only from his personal appearance and his nobility of character but also because he is a Giver of Laws in a Century of Fact-finding. Immediately contagious is the ever youthful *joie de vivre* with which he greets and communicates each of the discoveries made in the Lipschutz school. There have been many of these to illuminate the once dark continent of steroid and protein endocrinology which held so much of significance to medicine, cancer in particular.

The present volume is a succint but comprehensive account of the work of a pioneer in exceedingly difficult fields and it contains many suggestions, both overt and covert, which will interest all workers in the fields of endocrinology and of cancer. We will always be grateful for the sacrifices of Professor Alexander Lipschutz which made this book possible.

The Ben May Laboratory
for Cancer Research,
The University of Chicago.

December, 1956.

FOREWORD

By Professor ALEX HADDOW, M.D.

By far one of the most stimulating meetings at the Sixth International Cancer Congress, held at São Paulo in 1954, was that on "steroids and cancer." The main paper on the experimental side was given by Professor Lipschutz, and the present volume is, as he explains, a fuller version of that contribution. It provides us with a conspectus, in his own inimitable and enthusiastic style, of the most recent phases in an investigation which he and his school have conducted over no less a period than a whole generation. A main feature of this work was the induction, in the guinea-pig, of atypical hyperplasias, and tumours, through the experimental production of a hormonal imbalance, or disturbance of steroid homeostasis; and, particularly, the formation of uterine and extra-uterine abdominal fibroids due to prolonged treatment of the female guinea-pig with oestrogen. As to the mechanism, Professor Lipschutz has more recently, by employing the procedure of so-called "ovarian fragmentation," drawn attention to the importance of the irregularities thus induced in the sex cycle, and to the ovarian-hypophyseal imbalance which they then entail. Hence, both the uterine and extra-uterine atypical growth responses which he describes are very probably due primarily to a failure of ovarian function, and to a resultant intra-hypophyseal imbalance between the organotrophic hormones. Not the least interest of these growths lies in their varying degrees of oestrogen-dependence, and thus they comprise a group of conditioned neoplasms, the study of which, with the recent work of Furth, Foulds and others in similar directions, has done much for our understanding of the "progression" of tumours through various stages of hormonal dependence to the ultimate state of true autonomy.

Professor Lipschutz not only summarises the work and ideas of his own school, but also relates them to the contributions of many others in the field of endocrine carcinogenesis—of Bielschowsky, Biskind, Horning, Furth, Gardner, Lacassagne, and Mühlbock—to name but a few; and describes the application of

other agents than oestrogens (especially progesterone, testosterone and cortisone), and of other experimental procedures such as intrasplenic ovarian grafts, parabiosis and ovarian ligation, in the distubance of steroid homeostasis. Lastly he deals with a second discovery, namely the anti-fibromatogenic action of many steroids, and with questions of treatment whether through hormone administration or adrenal or pituitary ablation, with particular reference to the outstanding work of Huggins and the Chicago school.

Professor Lipschutz is one of the great worthies of cancer research, commanding not merely the respect of his colleagues but also their affection. His wide interests—and we also think of him as a scholar in South American anthropology—have in no wise hindered his making a characteristic and weighty contribution to the experimental study of tumours. It is a contribution for which we are grateful, and which will occupy a permanent and honoured place—when the history of the subject finally comes to be written—in one of the main chapters of cancer research.

Chester Beatty Research Institute
Institute of Cancer Research:
Royal Cancer Hospital.

February, 1957.

PREFACE

This booklet consists of an enlarged and up-to-date version of an address the author delivered at the end of July, 1954, at the Sixth International Cancer Congress held in São Paulo, Brasil. The session was dedicated to the problem of Steroids in Cancer. Professor Charles Huggins of Chicago was the speaker on the clinical aspects, and the author the speaker on the experimental aspects, Professor Alexander Haddow of London being in the chair. An ample discussion of all these problems also took place.

Our finding that atypical growth, or tumorigenesis, can be elicited in the guinea-pig by an experimentally induced hormonal imbalance due to an overthrow of the normal hormonal interplay between ovary and hypophysis dates from more than twenty years ago. More recently knowledge in this experimental field has been greatly enlarged thanks to the work of different authorities; results reached till the beginning of 1950 have been summarized in Part III of *Steroid Hormones and Tumors* (Baltimore, 1950). Thus, the present booklet refers only to work done between 1950 and 1956 and is to be regarded as an extension of Part III of the mentioned book. The reader will appreciate the amazing development experimental study of the pertinent problems has experienced in such a short time.

I have taken the liberty to suggest in my address also two lines of research which at the first glance must seem not sufficiently warranted by experimental facts: (1) the possible bearing of the so-called hypophysial reflexes for tumorigenesis, and (2) polypeptids in anticancer therapy. Needless to say that I am fully aware that both these suggestions are daring and provocative, and I should very much like to make it clear to the reader that these daring and provocative suggestions are brought forward only as such. But it happened that at the same moment when I was bringing forward in São Paulo the daring suggestion that environmental stimuli acting via the cerebral cortex on the hypophysis might be operative in what may be termed "gynaecological" tumorigenesis (section 15), Dr. Fels, a distinguished Argentine investigator, himself a gynaecologist, had already completed important experimental research which is much in favour of our suggestion: he was able to increase incidence of experimentally induced ovarian tumours in rats by prolonged exposure of the animals to light. And soon afterwards my attention was called to the feeling now prevailing among our colleagues in U.S.S.R. that in the origin of disease in general, greater interest than hitherto should be taken in centripetal

nervous stimuli, both internal and environmental, a feeling strengthened by the work of Pavlov and his followers.

Other readers may nourish the feeling that the idea of a deviation of the steroid homeostasis being at the root of some tumours could possibly be brought into connexion with the concept of stress as forwarded by Selye on the basis of outstanding experimental work done by himself and others in various fields of physiology and pathology. But this again, for the moment, cannot be but a stimulus for further experimental and clinical research.

It is useful to be always aware of the real place which corresponds to "suggestions" and "experimentally established facts" in the very frame of Medicine, because of the risk of making overhasty use, in therapeutics, of these two tools of medical thought, overhasty use by no means warranted by newly acquired experimental knowledge. Indeed, both suggestions and experimental knowledge with which we are dealing on the following pages should be of immediate "interest" not only for the research worker but also for the medical practitioner and especially the gynaecologist. But there is a difference between what is scientific interest for progress in experimental research the modern practitioner must, and is willing, to cultivate, on one hand, and practical application in patients, on the other hand.

The booklet includes also extracts from lectures delivered before the medical profession on behalf of the Faculty of Medicine of Universidad de Chile in 1953 in various towns of North and South in this country. Subsequently, in September and October, 1954, I was granted the opportunity to lecture on experimental cancer problems as summarized in the present booklet also in Peking, Shanghai and Tientsin under the auspices of the China Medical Association. My most sincere thanks are due to this institution and likewise to our Government and the National Health Service for facilities afforded for the trip. A lecture on the same topic was also delivered at the Institute of Experimental Cancer Research of the Academy of Medicine in Moscow. I greatly profited from discussions with my colleagues in China and Moscow.

Before closing this preface I should like to mention that the privilege of having been able to contribute more recently to knowledge on the steroid homeostasis and tumorigenesis I owed to the enthusiastic collaboration of various faithful associates and friends, especially Drs. Rigoberto Iglesias, Silvio Bruzzone, Elvira Mardones and others. Their recent work, still

unpublished or in course of publication, has considerably enlarged my own horizon.

I should like to thank also the linguistic help given to me by my wife and secretarial work done by Mrs. Dagmar Staden and Miss Yvonne Pivot.

My sincere thanks are due to Messrs. W. Heffer & Sons for the publication of the booklet. This is the renewal of an old friendship between Editor and Author. More than thirty years ago Messrs. W. Heffer & Sons were generous enough to publish my big book on the *Internal Secretions of the Sex Glands* which became the starting point for my work on tumours done in the last twenty years. With this book an amusing incident took place. While visiting a Medical School in a foreign country and being shown around by the Dean, the latter suddenly exclaimed: "What a curious coincidence of names! Twenty years ago when a student preparing for examinations I used a book on sex hormones—and the man who wrote it had just the same name as yours!" Well, I fear, fate will not favour me with a similar incident with the present booklet—in another twenty years time . . . .

A. LIPSCHUTZ.

Santiago de Chile,
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25th August, 1956.

CONTENTS

1. Steroids as Tumorigens

The bearing of steroid metabolism on cancerigenesis is only one of the many points of a broader problem, i.e. of steroid homeostasis and tumorigenesis, and of steroid homeostasis as a powerful means of antitumoural autodefence. It is with these special aspects that I should like to deal now. Indeed, I shall refer almost exclusively to experimental observations and not to clinical ones.

It is nowadays an established fact that tumoural growth, including cancer both of epithelial and conjunctive tissues, can be elicited in animals by the administration of oestrogen. An overwhelming wealth of oestrogen-induced tumoural structures variable according to the species has been reported: adenofibroma and adenocarcinoma of the mammary gland; adenomatous polyps of the uterine mucosa descending into the vagina; deep invasion of the myometrium by proliferating glands revealing the picture of adenomyosis or adenocarcinoma; cervical epithelioma and cancer; uterine adenomyoma; uterine fibroid and myoma; fibroid, or desmoid, of the abdominal serosa; fibroid of the thoracic serosa; metaplasia of the prostatic ducts; utricular fibromyoepithelioma of the prostatic region; tumour of the interstitial gland of the testicle; adenoma of the pituitary; adenoma of the kidney; leukemia; lymphosarcoma of the thymus; sarcoma of the lymphatic glands (summaries: Lacassagne, 1950, 1955; Lipschutz, 1950; Hertz, 1951; Dmochowski and Horning, 1952; Gardner, 1952, 1953, 1954; Gardner *et al.*, 1953; Burrows and Horning, 1952). Some of these tumoural structures acquire "autonomy" as time goes on, i.e. they continue to grow when oestrogen is withdrawn; some produce metastases; some become transplantable. However, most of these structures remain dependent upon the oestrogen by which they have been elicited.

Tumoural growth can be produced or enhanced also by the administration of progesterone as shown some years ago in work with mammary cancer in mice and rats (Symeonidis, 1948; Cantarow *et al.*, 1948; Jull, 1954a). Tumoural growth can be enhanced indiscriminately with oestrogen, testosterone and progesterone as recently shown in this laboratory with a spontaneous transplantable granulosa cell tumour in the rat (Iglesias and Mardones, 1954*b*, 1956*b*). Resistance against the taking of a tumour of an alien strain in rats, or of an alien species, can be overcome with the administration of cortisone (Foley and Silverstein, 1951; Foley, 1952; Toolan, 1953, 1954; Patterson *et al.*,

1954; Handler *et al.*, 1954). These are but a few though very striking examples drawn from experimental work.

On the other hand much experimental work has been done in the last 16 years on the antitumorigenic action of steroids (summaries: Lipschutz, 1950; Stock *et al.*, 1951). The bearing of structural particularities of steroids on their antitumorigenic action has been studied (Lipschutz *et al.*, 1951, 1952; Bruzzone *et al.*, 1951; Mardones *et al.*, 1952, 1953, 1954, 1956; Jadrijević, Mardones and Lipschutz, 1956). The results have been of use also to the clinician (summary: Lipschutz, Iglesias *et al.*, 1952). The amazing increase of potency obtained in the last years with various synthetic pregnene derivatives—Δ^{11} and 19-norprogesterone, Δ^{1} and 2-methyl-hydrocortisone, and especially those halogenated with fluor—may open quite unforeseen possibilities (see also Section 16).

However, the fact that steroid hormones *administered* to the body may produce tumours or enhance tumoural growth, and likewise the fact that tumoural growth may be prevented or interfered with by the administration of certain steroids—these facts by themselves do not yet prove that steroids have anything to do with what is supposed to be spontaneous tumorigenesis in the body. This statement which I am sure will be welcome to the clinician, seems to me paramount and fundamental.

What do we know about the tumorigenic and antitumorigenic actions of steroids *when not introduced from outside*, but secreted by the individual's own glands?

There is a classical proof in favour of the thesis that steroids secreted by the individual's own glands may elicit cancer, a proof known to everyone: the dependence of mammary adenocarcinoma upon oestrogen in certain strains of mice as evidenced by famous work of Loeb (Lathrop and Loeb, 1916; Loeb, 1919) and Lacassagne (1932). The study of this spontaneous cancer has greatly contributed to disentangle the riddle of tumoural growth in general—and at the same time to show its almost hopeless complexity (Dmochowski and Horning, 1952). However, after having studied extensively the genetical conditions so far as they refer to the hereditary reactivity of the mammary region in mice the authorities reached the conclusion that there is in the cancerous strain a genetically determined overproduction of oestrogen (Korteweg, 1948), or a hereditary abnormal hormonal constitution favourable to the development of breast cancer (Huseby and Bittner, 1948; Bittner, 1948, 1952, especially 1954, 1955; Ranadive and Khanolkar, 1948; Mühlbock, 1949, 1952).

Then came another striking proof: in the work of Huggins

prostatic carcinoma in man was shown to be dependent upon androgen, in any case in a long initial though already metastasizing phase of its growth (Huggins, 1941–1948).

More recently a third proof was added: Foulds showed that nodules of mammary adenocarcinoma which have grown during pregnancy may diminish and disappear after delivery (Foulds, 1952). Other authorities have made similar observations (see Haddow, Gardner, Dmochowski, in the discussion of the paper of Foulds). One cannot avoid thinking that here the tumoural conjuncture is dependent upon tumorigenic and antitumorigenic steroids produced, inactivated or eliminated at different levels in the various phases of reproduction.

A fourth proof must be mentioned: there are transplantable spontaneous tumours which take indiscriminately in 100 per cent of males or female mice, intact or castrated, but whose growth is influenced by the gonad. Mühlbock (1952*a*) when studying an ovarian tumour in mice found that it grew better in intact males than in castrated ones, and better in castrated males than in castrated females. The latent period was longer in castrated males than in intact ones. The transplantable spontaneous granulosa-cell tumour found by Iglesias in the AXC rat takes in 100 per cent of intact or castrated males and females but the latent period and survival are shorter in intact animals; the gonadal influence can be replaced quantitatively by gonadal steroids (Iglesias and Mardones, 1956*a*, 1956*b*). With the shorter survival, when the gonads are present or when gonadal steroids are administered, a smaller final weight of the tumour is reached; one must thus assume that the shorter survival is due to the malignancy of the tumour being enhanced by gonadal steroids (or reconditioned after each new transplantation). There can be no more spectacular demonstration of the dependency of autonomous and *malignant* tumoural growth on the steroids of the own body.

2. Ovarian-Hypophysial Imbalance in "Ovarian Fragmentation"

The above statements on the dependence of spontaneous cancerous growth upon steroids secreted by the individual's own glands seem to annul doubts as to the validity of experiments with tumours due to the administration of steroids. However, none of the mentioned four proofs gives any insight into the dynamics which make a tumorigenic steroid conjuncture or constellation originate in the body. Probably the first step as to this was made in our work of about thirty years ago with what we called "ovarian fragmentation" or subtotal castration in rabbits, cats and guinea-pigs. Those on the latter species were of an especial interest. Our experiments have been repeated by various other workers, and at the actual moment a rich harvest is available in this field (Lipschutz, 1925, Lipschutz and Voss, 1925; Lipschutz, 1931; Burch, Wolfe and Cunningham, 1932; Lipschutz, 1936–1938; Viñals, 1934; Osnowikoff, 1934; Morató, 1941; Nadel, 1949; Ponse and Dovaz, 1950, 1951; Bruzzone, 1951; Bruzzone and Lipschutz, 1954).

In guinea-pigs with ovarian fragmentation, i.e. with one ovary and the greater part of the second ovary removed, an irregular sexual cycle establishes itself; prolonged oestrus phases may be followed by prolonged anoestrus phases. The uterus may increase greatly up to the ten- and even twentyfold of its normal weight. There are also other evident signs of a prolonged oestrogenic action which deserve special attention in our context: there may be a cystic glandular hyperplasia of the endometrium, metaplasia of the latter, and even invasion of proliferated glands into the myometrium. Polyps of the uterine mucosa may fill the uterine cavity; there may be epithelioma of the uterine cervix; uterine fibromyoma, or fibroma of the mesentery.

In recently published experiments of Bruzzone in our laboratory the manifestations of tumoural growth two or four years after subtotal castration were extremely variable (Bruzzone and Lipschutz, 1954). There were several cases with subserous uterine adenomyoma or adenofibroma; there were vascular polyps; in other cases the whole uterine cavity was filled with adenocarcinomatous growth. A fibroid, or desmoid, of the intestinal serosa was present in one case. In an animal necropsied 45 months after operation there was a carcinoma of the renal pelvis originating from the mucosa. Nodules of Wolffian tubules, often cystic, near the tube, may occur. There were numerous

nodules seemingly of Wolffian origin in the abdominal cavity in a case described by Ponse and Dovaz (1950, 1951).

A nodular hyperplasia of the adrenal cortex also takes place (Bruzzone and Lipschutz, 1954).

The extraordinary variability of tumoural responses under these experimental conditions is very significant from the point of view of human pathology.

A first insight into the dynamics of these so manifold and variable findings in experiments with ovarian fragmentation has been won through the study of the ovarian fragment or remnant. It may contain luteic cysts sometimes filled with blood. There may be also haemorrhagic follicles. These findings give us a very important hint: an ovarian-hypophysial imbalance due to a primary failure of the ovarian function is evidently the starting point of the described uterine and extrauterine tumoural response. We assumed about twenty years ago that the ovarian remnant loses the faculty to control duly the gonadotrophic function of the hypophysis. To examine this question we injected the hypophysis of operated guinea-pigs into infantile mice; there was a greater increase of uterine weight than with the hypophysis of normal guinea-pigs (Lipschutz, 1936*a*). Experiments with the administration of the hypophysis of animals with ovarian fragmentation were not numerous; but they were sufficient to convince us that we were on the right way when reaching the conclusion that a tumoural response can be elicited when the normal ovarian-hypophysial balance is experimentally upset. There may be Wolffian nodules in the ovary itself (Lipschutz, 1938). There may be local hyperplasia of the germinal epithelium (Bruzzone and Lipschutz, 1954). As already said, the first step in the sequence of events is evidently the *primary* functional failure of the ovary; a hypophysial imbalance is the immediate *secondary* result; an abnormal follicular development is established and the Law of the Sexual Rhythm is transgressed.

Our findings in the guinea-pig were amply corroborated by the authorities. More recently similar observations have been made also in mice (Hummel, 1954; see also Sect. 10).* On the contrary, in Rhesus no disturbance of the sexual rhythm was obtained with ovarian fragmentation (Dahl-Iversen *et al.*, 1942). However, cystic glandular hyperplasia of the endometrium has been reported in a woman after removal of an entire ovary and part of the second (Cruz, 1935).

* Work with two strains of mice is in progress in our Institute (Mardones and Lipschutz).

3. The Law of the Sexual Rhythm

We have referred to the overthrow of the Law of the Sexual Rhythm; its fundamental importance for the understanding of the tumorigenic action of oestrogens has been evidenced in experiments very easy to reproduce. Abdominal fibroids in the guinea-pig can be produced only by the *continuous* action of oestrogen, i.e. when the Law of the Sexual Rhythm is transgressed. Then quantities of oestrogen exceedingly small compared to those formerly used in similar experiments, are already sufficient to elicit the mentioned tumoural response; the same quantities of oestrogen administered intermittently failed to produce abdominal fibroids (summary: Lipschutz, 1950). This was an impressive result. Nature would not have been able to make use of oestrogens in mammals without recourse to the Law of the Sexual Rhythm, be it by a rhythmic decrease of production of oestrogen, or by rhythmic production of progesterone which antagonizes oestrogenic action.

One cannot but feel inclined to apply these findings and concepts to oestrogen treatment of women in the menopause. One may take them as a warning against the continuous use of oestrogens in the menopause. Discontinuity of oestrogen administration, i.e. "Cyclical Estrogen Therapy," has been claimed (Palmer, 1942; Lipschutz, 1950, ch. 23).

Since tumoural responses may be elicited by an overthrow of the Law of the Sexual Rhythm it is evident that better knowledge of the mechanisms which underlay the sexual rhythm in mammals would be fundamental also for an understanding of the origin of tumours including cancer in the body of women especially of the uterus and breast. What do we know about these mechanisms? When summarizing present knowledge in this still questionable field of experimental research one becomes aware how important further progress in this special physiological domain would be for the understanding of many problems of tumorigenesis.

More than thirty years ago, in work with guinea-pigs, the conclusion was reached that the sexual rhythm is not inherent to the ovary and that it depends on some extragonadal factor present in the female but absent in the male. Our conclusion was based on sound experimental facts.

In classic work of Steinach (1912) with ovarian transplantation into castrated male guinea-pigs mammary development comparable to that of lactating females was obtained. Steinach called this condition "feminization." But "*hyper*feminization" seemed to us to be a more appropriate name for the condition experimentally