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COMMITTEE ON ARRANGEMENTS

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PREFACE

From September 4th to 9th, the 1960 meeting of the Laurentian Hormone Conference was held at Mont Tremblant, Quebec. The chapters of this book contain the fourteen papers delivered to that meeting as well as the discussion that followed after each paper.

The contents of each volume of *Recent Progress in Hormone Research* are testimony to the objectives of the Laurentian Hormone Conference. The Committee on Arrangements each year attempts to have presented for thorough discussion significant contemporary developments in experimental endocrinology. The papers in this volume pertain to five active hormone research areas, i.e., those concerned with testis function, aspects of reproduction, aldosterone secretion and activity, neurohypophyseal physiology, and hormonal regulation of aspects of carbohydrate metabolism.

The testis with its dual role of spermatogenesis and androgenesis in turn finds itself under dual control: by hypophyseal hormone and genetic factors. The role of the latter in hormone secretion is receiving increasing attention, and finds full treatment in the paper by Drs. Nowakowski and Lenz.

The section concerned with hormones and reproduction covers a wide field, ranging from the effects of erucic acid and its congeners upon gametogenesis and gonad function to the specific role of corpus luteum hormone in electrolyte regulation. In Dr. Nalbandov's paper, we have a much-needed review of significant investigations in recent years of domestic animals' endocrinology, with special emphasis on hormonal factors in reproduction. The two papers concerned specifically with hormone metabolism and transport in fetal life illustrate the importance attached to increased factual knowledge of the endocrinology of pregnancy and neonatal life.

The papers in the aldosterone section illustrate the extremely lively interest in this essential corticosteroid. The once apparently simple problem of the regulation of aldosterone secretion is clearly presented in its complexity by Dr. Davis. The study of the role of aldosterone in experimental nephrosis points up also certain complexities, ranging from metabolic transformations to specific hormone effects. The now classic clinical involvement of the hormone is thoroughly reviewed by the discoverer of hyperaldosteronism and his colleague, Dr. Louis.

That electrolyte regulation has a varied hormonal basis is obvious not only from Dr. Landau's paper, and in those concerned with aldosterone, but also in the section concerned with neurohypophyseal hormones. The review by Dr. Sawyer and the specific permeability studies reported by Dr. Leaf and Dr. Hays point up the numerous involvements of these hormones in body, organ, and tissue metabolism.

In the final section, a classic problem, hormonal factors in glucose uptake, is examined in the light of recent developments by Dr. Park. In turn, Dr. Miller examines experimental data on aspects of organic metabolism in

the liver, a long recognized target organ for hormone action now made much more accessible by modern techniques.

In considering the program herein presented, we are constrained to remark on the striking diversity of problem and method in hormone research. The production and release of hormones, their transport, their metabolic transformations, their mode of entry into target organs and tissues, their varied specific effects, their involvement in the metabolism of energy-producing systems, in normal and abnormal growth, in embryogenesis and development concern practically all of the phenomena attendant on being alive. To characterize and delineate the hormone-involved processes, the experimentalists call upon such varied techniques as radioisotopy, microchemistry, histochemistry, organ perfusion, experimental surgery, biometrics, and upon all the resources of clinical observation. To have these problems and techniques presented by the experts devoted to them is the objective of this and every volume of *Recent Progress in Hormone Research.*

Acknowledgments

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The Committee on Arrangements is indebted to Drs. A. Bongiovanni, R. O. Greep, J. R. K. Preedy, A. Segaloff, J. F. Tait, G. Liddle, and J. Russell for acting as chairmen for the sessions. Mrs. Jacqueline C. Foss and Mrs. Mina Rano acted as secretaries to the Conference, and Mrs. L. P. Romanoff kindly prepared the subject index of this volume.

Shrewsbury, Massachusetts GREGORY PINCUS
June, 1961

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I. TESTES FUNCTION

Function of Normal and Abnormal Testicular Interstitial Cells in the Mouse[1]

ROBERT A. HUSEBY, OSCAR V. DOMINGUEZ, AND LEO T. SAMUELS

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The function of normal and of abnormal testicular interstitial cells in many species has been the subject of multitudinous investigations since the birth of endocrinology as a science. It is not the purpose of this presentation to review these massive data. Rather, we wish to present some of the data obtained in our own laboratories over the past few years that deal with the production of interstitial cell testicular tumors in mice, the function of these tumors, and the influence of certain endocrinological manipulations upon the histology and function of nonneoplastic interstitial cells in the mouse. Specific reference to data obtained by others will be limited to those that appear to be most pertinent to these particular studies and wherever possible will include those publications that review literature in some detail.

I. *In Vivo* STUDIES

1. Estimation of Testicular Interstitial Cell Function

It is fortunate that, in the mouse, androgens produce recognizable changes in several tissues; those occurring in the seminal vesicle-coagulating gland complex, the submaxillary salivary glands, and the kidneys being the most readily recognizable. Reasonable quantitation *in vivo*, however, is often difficult or even impossible. The response of the seminal vesicle-coagulating gland complex seems to be quantitative over a considerable range of androgen stimulation and seminal vesicular size (the upper limit approaching that seen in normal intact males) when more chronic assay procedures are used—carried out over 10–14 days rather than the customary 3–4 days.

[1] These investigations were supported by grants-in-aid given to the University of Colorado Medical Center by the Damon Runyon Memorial Fund for Cancer Research, Inc. (DRG 333) and the National Institutes of Health (C-3950), to the American Medical Center at Denver by the National Institutes of Health (C-5191), and to the University of Utah College of Medicine by the American Cancer Society, Inc. (T12 and T20) and the National Institutes of Health (C-307).

[2] A division of the Eleanor Roosevelt Cancer Research Foundation.

Although there is quite a bit of disagreement particularly in the older literature [for reviews see (2, 25, 32)] as to whether the various sex steroids exert antagonistic actions one toward the other, it does appear that, under certain circumstances at least, the response of the seminal vesicle to administered androgen is materially altered by the presence of other hormones. As illustrated in Table I, the concomitant administration of a relatively large dose of diethylstilbestrol, a dose that by itself causes extensive squamous

TABLE I

Effect of Various Doses of Stilbestrol and of Stilbestrol plus Progesterone upon the Response of the Seminal Vesicle-coagulating Gland Complex (S.V.) and of the Left Kidney to Administered Testosterone Propionate (TP)[a]

Treatment	Number of animals	Body weight (gm.)	S.V. weight (mg.)	Left kidney weight (mg.)
Controls, no Rx	12	23.4	26.6	153.0
Control + 16.7 μg. TP	12	24.1	94.7	176.0
Control + 50 μg. TP	12	24.9	176.0	198.0
0.5 μg. Stilbestrol alone	12	21.8	31.2	140.3
0.5 ug. Stilbestrol + 16.7 μg. TP	17	23.3	101.0	178.0
0.5 μg. Stilbestrol + 50 μg. TP	18	23.1	177.0	189.0
10% Stilbestrol	6	23.1	43.2	153.2
10% Stilbestrol + 16.7 μg. TP	11	23.7	77.3	161.4
10% Stilbestrol + 50 μg. TP	12	25.2	116.3	191.6
0.5 μg. Stilbestrol + 2.5 mg. progesterone	12	26.8	34.6	160.0
0.5 μg. Stilbestrol + 2.5 mg. progesterone + 16.7 μg. TP	11	27.4	75.8	173.0
0.5 μg. Stilbestrol + 2.5 mg. progesterone + 50 μg. TP	12	26.0	121.8	169.5

[a] BALB/c mice 6–10 weeks of age were castrated, and the TP in 0.05 ml. of oil was injected every other day beginning the day of surgery for 8 injections. The animals were sacrificed on the sixteenth postoperative day. The 0.5 μg. stilbestrol was administered orally in the diet, the 10% stilbestrol as a fused pellet with cholesterol (approximate weight of each pellet = 10 mg.) was implanted subcutaneously, and the progesterone was injected daily as an aqueous suspension.

metaplasia of the coagulating gland epithelium in castrate males, materially reduces the response of the seminal vesicle-coagulating gland complex to administered testosterone proprionate. The administration of a smaller dose, one that does not cause squamous metaplasia of the coagulating gland epithelium, does not seem to interfere. Progesterone in large doses also has been shown to act as an antiandrogen in chicks and in rats (26, 27) and in high dosage appears to have the same qualities in mice.

Androgenic compounds have been shown to bring about a hypertrophy of the renal cortex in the mouse and to cause the usually squamous parietal cells of Bowman's capsule to become columnar in configuration (30). The latter is helpful qualitatively in recognizing androgen stimulation but is of little value from a quantitative standpoint, whereas the former under certain circumstances might be of some value quantitatively. Although the renal hypertrophic effect of testosterone proprionate does not appear to be altered by the presence of relatively large amounts of estrogen, the slope of the response curve is rather flat, making this an insensitive assay. We have not carried out extensive studies of the time required to achive maximal kidney hypertrophy, but it is our impression that a maximal response takes longer to occur than does that for the seminal vesicle-coagulating gland complex and actually has been rather inconsistent in our assays carried out over 10–14 days. This fact, coupled with the fact that the absolute weight of the kidneys can be altered by other factors such as diet and hypophysectomy, greatly limits the usefulness of this response in quantitative studies. It is of interest that the antiandrogen effect of progesterone appeared, in one study at least, to reduce the response both of the seminal vesicle and of the kidney to administered testosterone.

The changes that occur in the submaxillary salivary gland give only very rough quantitation. These changes consist of an increase in weight and an accumulation of secretory granules in the apical portion of the cells of the tubular elements of the gland in response to androgen stimulation (21). This response does not appear to be materially affected by the presence or absence of estrogens, but is diminished considerably in the hypophysectomized animal (22). With increasing degrees of androgen stimulation, however, the accumulation of granules increases so that, upon histological examination of the glands, one does get some impression of the degree of androgenic stimulation. Nevertheless, since in adult castrates of either sex granulation persists to some degree in the tubular cells, minimal androgen stimulation has, in our hands, been very difficult to evaluate.

The histological appearance of the interstitial cells themselves seems to give us a rough indication of their function, at least in many circumstances. Normally functioning testicular interstitial cells have a characteristic morphology (Fig. 1). The nucleus is generally regularly spherical, or only slightly ovoid, has a relatively fine nuclear pattern with most of the chromatin near the nuclear membrane, and contains one and sometimes two rather prominent nucleolar bodies. The cytoplasm is moderately abundant, stains eosinophilic and in the usual hematoxylin-eosin preparation contains many fine, small, clear vacuoles giving it a finely granular appearance. After hypophysectomy,

the nuclear size decreases and the chromatin condenses, often taking on a "wheel cell" pattern (23). The cytoplasm decreases in amount, and the fine vacuolation disappears, as does the rather intense eosinophilia (Fig. 2). Within 2 weeks of the operation, small granules composed of a wax-like material, commonly referred to as ceroid, appear within the cytoplasm of the interstitial cells. This granulation increases in amount so that by 2 months after hypophysectomy the intertubular tissue is filled with fairly large cells whose cytoplasm is greatly engorged with this ceroid material. The majority of these cells retain a single nucleus whose chromatic pattern is similar to that of interstitial cells a week or so after hypophysectomy, but many of the largest cells are found to contain several nuclei without this characteristic chromatin pattern.

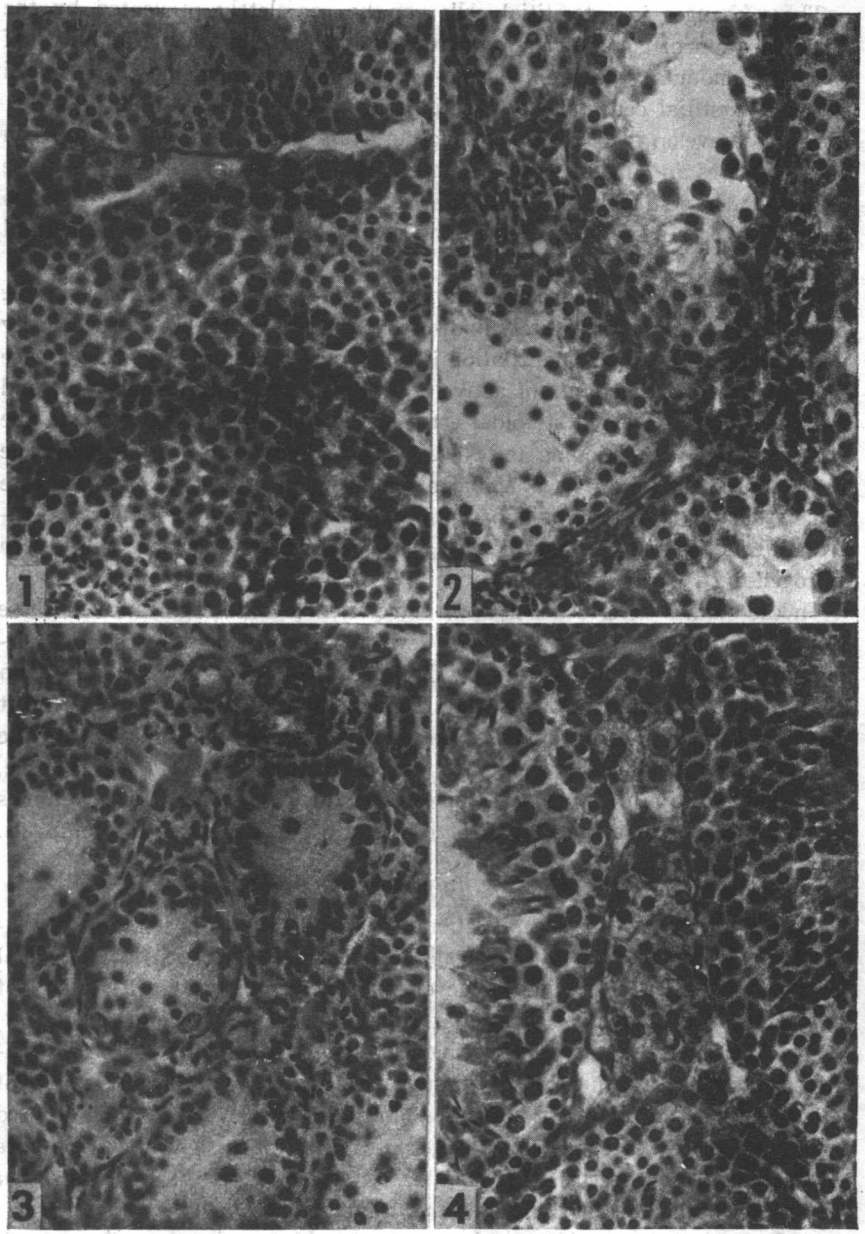
The amount of ceroid increases moderately, as does the number of large multinucleated cells, between the second and fifth months following the operation (Fig. 3); we have not continued our studies for longer periods. There seems to be little doubt that the ceroid pigment is of interstitial cell origin; however, whether its presence within an interstitial cell always reflects solely a decrease in function of that cell cannot be said with certainty at present. It is true, nonetheless, that an increase in pigment has been described in several situations, such as advancing age and vitamin deficiency, in which hypofunction of the interstitial cells would be expected, and in the mouse treatment with large doses of testosterone results in nuclear changes similar to those seen after hypophysectomy and ceroid pigment does accumulate within the cytoplasm.

FIG. 1. Section of a testis from a young adult BALB/c mouse containing two clumps of interstitial cells. The nuclei are generally slightly ovoid with a fine chromatin pattern and a prominent nucleolar body. The cytoplasm is moderately abundant, stains eosinophilic, and has a finely granular appearance. Magnification: × 300.

FIG. 2. Section of a testis of a BALB/c mouse 1 month after hypophysectomy. The nuclei of the interstitial cells are small with a condensed chromatin pattern. The cytoplasm has decreased in amount and is no longer intensely eosinophilic; special stains demonstrate the presence of ceroid granules. Magnification: × 300.

FIG. 3. Section of a testis of a BALB/c mouse 5 months after hypophysectomy. The nuclei of the interstitial cells have remained small. The cytoplasm has, in general, increased in amount and has a yellow appearance in the usual hemotoxylin and eosin preparation. Special stains reveal the cytoplasm of most of these cells to be engorged with ceroid pigment. Magnification: × 300.

FIG. 4. Section of a testis of a BALB/c mouse 1 month after the implantation of a 10-mg. fused pellet containing 10% stilbestrol in cholesterol. In general the nuclei are similar to those of untreated control males of the same age. The cytoplasm is increased in amount, contains many clear, rather large vacuoles, and special stains fail to reveal significant amounts of ceroid. Magnification: × 300.



These changes in interstitial cells can be completely prevented by the administration of gonadotropic preparations having luteininzing activity. If excessive amounts of this gonadotropic hormone are administered, the nuclei of the interstitial cells tend to enlarge and become somewhat less regular; they may develop a peculiar intranuclear hyaline inclusion. The cytoplasm also increases in amount and becomes more vacuolated. Although these changes in interstitial cell morphology are not readily quantifiable they do seem to give us some indication of the degree of function of the interstitial cells.

Other aspects of testicular function are most difficult to evaluate in the mouse. Although it is definitely known that the testes produce estrogenically active compounds, their quantitation is significantly obscured by the presence of relatively large amounts of active androgens. Thus, the mammary gland response to administered steroidal estrogens is significantly depressed in the face of actively functioning testes (2). The administration of testosterone propionate itself results in a significant enlargement of the uterus in castrate female mice, and androgens apparently exhibit both inhibitory and cooperative effects on the vaginal mucosa when administered concurrently with estrogens (20).

In the studies described below we have tried to take cognizance of these difficulties and wherever possible to develop an experimental design to circumvent them, even though this has imposed a considerable restriction upon the types of experiments carried out. In some of the data to be presented it has been impossible to obviate possible changes in the function of the animal's own pituitary secondary to various treatments, since, for example, the administration of large doses of estrogen to hypophysectomized animals generally results in an excessively high mortality in the experimental animals.

2. *Factors Affecting Interstitial Cell Tumorigenesis*

It had been shown in several laboratories that the administration of relatively large amounts of estrogenically active substances, be they steroidal or nonsteroidal, resulted in the development of interstitial cell tumors of the testes in a number of different strains of mice [for reviews see (1, 12, 15)]. The precise mechanism by which this was effected had not been elucidated, although Gardner (11) had suggested that it might be the result of an overstimulation with pituitary gonadotropins secondary to and initiated by the estrogen treatment. Since large doses of estrogen significantly obscure evaluation of the precise endocrine status obtaining in the animals, other means of producing this type of tumor were sought.

It had been shown that ovarian grafts would function in male animals

whose testes were confined within the abdominal cavity (32), so we employed this experimental design in our first approach to the problem. When interstitial cell tumors developed in a high percentage of BALB/c mice that were rendered cryptorchid and implanted subcutaneously with a pair of ovaries

TABLE II

Development of Interstitial Cell Tumors of the Testes in BALB/c Mice with Various Treatment Regimens[a]

Treatment	No. tumorous/ no. animals	Average tumor age (months)	Seminal vesicular size after 3 months' treatment	Interstitial cell morphology after 3 months' treatment
10% Stilbestrol	12/14 (86%)	11.5	Less than 50% of normal (squamous metaplasia)	Abnormal hyperplasia with ceroid accumulation
Cryptorchid with transplanted ovaries	27/35 (77%)	19.3	Less than 50% of normal (variable)	Slightly atrophied
Cryptorchid on stilbestrol diet	18/22 (82%)	20.1	80% of normal	Essentially normal
Stilbestrol diet only	8/24 (33%)	19.2	80% of normal	Essentially normal
Cryptorchid only	7[b]/15 (47%)	22.9	Normal	Hypertrophied without ceroid accumulation
Intact with transplanted ovaries	0/12	—	Normal	Normal
Intact controls	0/50	—	Normal	Normal

[a] All treatments were begun when the mice were 4–6 weeks of age and were continued throughout their lives until palpable tumors developed or the animal was sacrificed because of poor physical condition. The 50 untreated control animals lived to an average age of 21.2 months, and at autopsy no gross tumors were seen. The testes of half of these were examined histologically and failed to reveal any evidence of hyperplasia or tumor formation.

[b] All these tumors were of small size; none enlarged the small cryptorchid testes.

(17), the effects of small doses of stilbestrol on tumor development in normal and in cryptorchid testes were investigated (Table II). It became evident from these studies that the dosage of estrogen and the position of the testes were both significant factors in interstitial cell tumorigenesis. However,

evaluation of androgen production by the testes and of interstitial cell morphology failed to yield evidence to support the thesis that excessive stimulation with pituitary gonadotropins played an all-important role in this process. Investigations were, therefore, undertaken to elucidate the effects of estrogenization and of cryptorchidism more completely.

3. *The Effects of Estrogen Administration on Interstitial Cells*

Although the gonadotropin-suppressive effects of large doses of estrogen have been described by many, the histological alterations that occur in the interstitial cells of the testes of the mouse after the administration of large doses of stilbestrol are entirely different from those seen after hypophysectomy or the administration of large doses of testosterone propionate. Within 2–4 weeks of the subcutaneous implantation of a 10-mg. pellet containing 10% stilbestrol fused with cholesterol, many of the interstitial cells are distinctly larger than normal as a result of increase in cytoplasm (Fig. 4). This cytoplasm in the usual hematoxylin and eosin preparation appears to be heavily vacuolated and with special stains is seen not to contain ceroid pigment. The nuclei remain essentially normal in appearance. In spite of the fact that the seminal vesicles are small and histologically show relatively little evidence of androgen stimulation, the weight of the testes is not diminished and spermatogenesis is well maintained.

After 2 months of treatment two cell types appear in the intertubular tissue: one, the large interstitial cells with excessively vacuolated cytoplasm, the other, small cells with a nuclear pattern similar to that seen in interstitial cells soon after hypophysectomy. In the cytoplasm of some of these small cells, ceroid is beginning to accumulate. By the third month the amount of intertubular tissue has definitely increased with the beginning formation of small nodules that are composed of the large interstitial cells, the smaller cells, some containing ceroid, and an occasional multinucleated cell filled with ceroid. As time goes on the number of large multinucleated ceroid cells increases throughout the testes and the large interstitial cells with frothy cytoplasm proliferate eventually to form macroscopic nodules and, finally, frank tumors.

Many of these changes can be prevented by the concomitant administration of ovine pituitary luteinizing hormone (LH) preparations. With such combined stilbestrol-LH treatment the morphology of the interstitial cells does not appear significantly different from that of interstitial cells of non-estrogenized mice treated with the same preparations. Neither the small cells with condensed nuclei nor the ceroid-containing cells accumulate, and the cytoplasm of all the interstitial cells remains very eosinophilic, with the

usual granular appearance. However, after 2 months of combined treatment in which the Armour preparation No. 227-80 was used, a distinctly exaggerated proliferation of these interstitial cells became evident. This proliferation continued until, by the fourth month of combined treatment, the testes were composed principally of interstitial cells that had crowded out the tubules, resulting in a great degree of atrophy of these structures.

This excessive proliferation in response to combined LH-stilbestrol therapy strongly suggests that stilbestrol exerts an effect directly upon the interstitial cells, altering their response to pituitary gonadotropin. Unfortunately to date it has been impossible to prove this conclusively by this experimental design since the ovine LH preparation originally employed is no longer available. The preparation made available to us through the Endocrinology Study Section of the National Institutes of Health (N.I.H.) does not appear to have as marked an effect, in this regard at least, as did the original Armour preparation No. 227-80. However, experiments carried out in hypophysectomized animals using the N.I.H. ovine LH preparation show that the degree of interstitial cell proliferation obtainable in combined stilbestrol-LH treated hypophysectomized animals is as great as that seen in the nonhypophysectomized controls treated in a similar manner and is definitely in excess of that seen with the administration of the N.I.H. preparation alone.[3]

We have not yet been able to establish with certainty whether or not this increased proliferation of interstitial cells significantly alters the rate of malignant transformation, but from our experiments to date it would appear that it does not. We have carried out a number of experiments in which testes of animals treated with stilbestrol and with stilbestrol plus LH for periods of time from 2 to 4 months were transplanted subcutaneously into castrate BALB/c males bearing 10% stilbestrol pellets. With this experimental design, interstitial cell tumors developed at the site of implant more readily when the implants were from mice treated with stilbestrol for 3 or 4 months than when they were from normal untreated animals. However, testes from mice receiving the combined LH-stilbestrol treatment showed

[3] In more recent studies, administration of the N.I.H. preparation at a level of 0.1 mg. per mouse per day (the dose of Armour's No. 227-80 that had been employed) was continued for 4 months, but no greater proliferation of interstitial cells than seen after 2 months of treatment was evident whether the stilbestrol-treated mice had been hypophysectomized or had intact pituitaries. Nevertheless this N.I.H. preparation appears to be as effective as the Armour one in eliciting androgen production by the testes of hypophysectomized mice in the dose range from 2.5 to 15 μg. per day. An explanation of this difference in the two ovine LH preparations is not immediately evident.

no greater tendency to form subcutaneous tumors than did those from mice receiving stilbestrol alone.

Once interstitial cell tumors develop as a result of intense estrogenization, it has been shown in several laboratories that many remain dependent upon estrogen stimulation for their continued growth (1, 10). Over the past few years we have been studying several aspects of the hormone dependency of a number of lines of transplantable interstitial cell tumors. In the course of these studies it has become evident that the degree of dependency frequently decreases as serial transplantation is continued, that the growth of the less dependent tumors can be influenced by a number of different endocrinological manipulations, and that different tumor lines respond to many of these manipulations in quite different ways. In spite of this complexity, it was found that the growth of all tumor lines in early transfer generations was augmented by estrogen administration and that all tumor lines grew with maximal rapidity when transplanted into intensively estrogenized recipients. This suggested the possibility that here too estrogen might exert a direct proliferative effect upon these abnormal neoplastic interstitial cells.

To test this possibility, two lines of tumors that had retained a high degree of hormone dependence were employed. Bits of tumor were implanted both in the subcutaneous tissues and within the substance of the spleen of the same recipients, and fused estrone-cholesterol pellets were then placed adjacent to the intrasplenic grafts. By this means it was hoped that the intrasplenic grafts would be bathed in estrogen while, owing to hepatic inactivation of the estrone released into the portal circulation, the subcutaneous grafts would be in an essentially estrogen-free environment. As controls, animals were employed in which fused cholesterol pellets were substituted for the estrone-containing ones. As can be seen from the data presented in Table III, the tumor grafts that were in juxtaposition to an estrone pellet grew in 15 of 22 animals, while growth did not occur in any of the subcutaneous grafts or in any of those placed next to the pellets containing cholesterol only. Thus it would appear that estrone did exert a proliferating effect directly upon the tumor cells, an effect that was not mediated by the pituitary. This does not mean, of course, that estrone per se in the total absence of pituitary hormones could maintain the growth of these tumors, but it does indicate that estrogens do affect directly not only normal, but neoplastic interstitial cells as well.

4. Effects of the Intra-abdominal Position

The inhibition of spermatogenesis and the degeneration of the germinal epithelium resulting from the increase in environmental temperature that

occurs when the testes are confined within the abdominal cavity are well-known phenomena. Less widely appreciated, and as yet poorly understood, are the endocrine changes that accompany this condition. Early in experimental endocrinology, it was shown that the cryptorchid testis continued to produce androgens at an essentially normal level as judged by maintenance of the secondary target organs. In fact, in the mouse, the weight of the seminal vesicle-coagulating gland complex is slightly, but definitely, increased when young adult males are rendered cryptorchid, and this level of androgen production is maintained for at least one year. The interstitial cells of the cryptorchid mouse testis also appear larger than those of normal

TABLE III

The Direct Effect of Estrone upon the Growth of Two Different Lines of Transplantable Testicular Interstitial Cell Tumors[a]

Tumor	Intrasplenic cholesterol		Intrasplenic estrone	
	Positive	Negative	Positive	Negative
C♂ 4658	0	6	9	2
C♂ 157220 line B	0	12	6	5
	0	18	15	7

[a] All recipients were intact male animals implanted with tumor both subcutaneously and intrasplenically with fused pellets placed next to the intrasplenic implant. The grafts were considered as "positive" if they contained areas of morphologically typical tumor cells. None of the subcutaneous grafts were "positive," being composed entirely of ceroid filled cells and small atypical nonproliferating tumor cells.

scrotal testes. Their cytoplasm is increased in amount and appears more extensively vacuolated, similar to the cytoplasm of interstitial cells of mice receiving large doses of estrogen (Fig. 5).

In spite of this continued production of androgens, it was repeatedly demonstrated that ovaries when grafted into cryptorchid males produced detectable amounts of estrogens, whereas similar grafts in males with normal scrotal testes appeared to be essentially nonfunctional. These changes could best be explained on the basis of an increased elaboration of pituitary gonadotropins associated with cryptorchidism and, in fact, Evans and Simpson (9) demonstrated an increase in gonadotropic content in pituitaries of cryptorchid male rats. Witschi *et al.* (33) demonstrated that an increased level of circulating gonadotropin followed destruction of the germinal epithelium by X-irradiation, though here too, androgen production was not diminished. Data were also presented from several laboratories suggesting that in castrate males replacement therapy with androgens at a level