Extensive investigations in different laboratories have resulted in many contributions to the question of the pathogenesis of mammary gland cancer in mice. This tumor occurs under only two conditions: (1) "hereditary predisposition," many workers having established (Tyzzer, J. A. Murray, Slye, etc.) that by proper selection strains of mice can be bred in which mammary carcinoma occurs in a definite proportion of the females, ranging from 100 per cent in some strains to none in others; (2) hormonal stimulation of the mammary gland. This latter effect has been attributed to folliculin for the following reasons. (a) In strains highly susceptible to mammary cancer the tumors occur spontaneously only in the females. (b) If, however, the latter are completely deprived of estrogenic activity by castration before the establishment of ovarian function, they remain cancer-free (Lathrop and Loeb, 50: Cori, etc.). (c) When subjected to continuous estrogenic therapy, males of these high-cancer strains also develop mammary cancer, the incidence sometimes being as high as 100 per cent (Lacassagne, 36; Bonser, Burrows, Cramer, etc.).

The mechanism of the action of folliculin has been widely discussed. We shall examine first the arguments for and against its rôle in the production of mammary cancer. Later we shall discuss the question whether certain pituitary hormones may not also play a part. Finally, attempts to prevent mammary carcinoma by means of the male hormone will be considered.

Rôle of Estrin in the Development of Mammary Cancer

In attempts to explain the occurrence of mammary cancer in males subjected to prolonged treatment with estrin, the following alternatives have been suggested. Either the hormone directly stimulates the mammary cells to cancer production or, by setting up the necessary anatomical substrate in the developing male mammary gland, it permits the hereditary predisposition to the disease to come into full play. The problem is still unsolved, not only for induced cancer in males, but also for spontaneous cancer in females, which may similarly be attributed either to the direct carcinogenic action of the hormone or to its stimulative action upon the mamma.

(1) Facts Favorable to the Idea of a Direct Influence of the Hormone in Cancer Production: (a) Two discoveries in 1933 led to the acceptance of a chemical theory of the pathogenesis of mammary cancer: first, the discovery

1 Read before the Third International Cancer Congress, Atlantic City, New Jersey, Sept. 11–15, 1939.

2 The author takes this opportunity to express his thanks to all those in the United States who have aided him in pursuing his researches, and for the facilities placed at his disposal by the Rockefeller Foundation, Mr. Alfred Busiel, Mrs. Blanche and Mr. Morris Nelson.
by Cook and Dodds of the estrogenic properties of certain carcinogenic hydrocarbons of molecular structure more or less related to that of the female hormone; second, that of Wieland and Dane, and of Cook and Haslewood, that methylcholanthrene could be prepared from acids derived from the bile.

It is tempting, by analogy, to attribute to estrin or to some one of its derivatives a carcinogenic property capable of acting upon the cells of the mammary ducts, as the hormone is known to be in contact with these, having been found in the milk (Courrier) and in the colostrum (Lacassagne and Nyka, 45).

(b) With this hypothesis experimental findings are in accord. In certain strains of mice, for instance, in which mammary cancer occurs spontaneously in only a very small proportion of the females, the normal rate of cancerization has been increased by estrin injection to 60 per cent in both sexes (Lacassagne, 37; Gardner, et al).

(c) Cancers other than mammary adenocarcinomas have also been observed, though much more rarely, in mice subjected to the prolonged action of estrogenic substances. These tumors, only exceptionally met with in normal animals, have been found in various strains in some of which no spontaneous cancer had occurred for a number of years. The most characteristic of these neoplasms is the epidermoid epithelioma of the uterine cervix (Lacassagne, 38; Loeb, Burns, et al). The relation between the morphology of these tumors and the estrogenic treatment is evident. The epithelium from which the cancerous process originates, through the influence of the hormone, first undergoes a metaplasia, which is maintained by prolonged treatment. Gardner, Allen, Smith and Strong have succeeded, by transplantations in series from animal to animal, in proving the cancerous nature of certain of these epithelial growths developing after injections of the estrogenic hormone.

(d) Finally, an important observation has recently been reported by Geschickter. Though it is well known that spontaneous mammary adenocarcinoma is as rare in the rat as it is frequent in the mouse, he was able to obtain, by the action of estrogenic substances, a considerable number of mammary adenocarcinomas both in male and in female rats of a selected strain in which since 1934 no spontaneous cancer had been registered in a colony of 2000 animals.

(2) Facts Unfavorable to a Direct Influence of the Hormone in Cancer Production: In contradistinction to the arguments enumerated above, certain observations favor the second hypothesis, i.e., that estrin is only an incidental agent, permitting that degree of development of the mammary glands which is necessary for the manifestation of a predisposing factor to cancer.

(a) The fact remains that no one has ever succeeded in producing a cutaneous epithelioma by the repeated application of the estrogenic hormones. Those investigators who have introduced estrin into the organism of the mouse by painting with benzene or chloroform solutions, and have thus succeeded in producing mammary carcinomas in males, have made no mention of any epidermal proliferation following this treatment. To this it may be replied that the hormone is able to produce cancer only in cells on which it exerts a physiological action.

(b) Much more important is the fact that it has been impossible, up to
the present, to produce adenocarcinoma of mice in strains not subject to mammary cancer. Prolonged experiments on numerous animals of several selected strains have all been negative (Lacassagne, 41). The rare cases published by some authors, as Bonser, Stickland and Connal, have occurred only in females, and it is far from certain that the strains to which these mice belonged were absolutely free of spontaneous cancer.

(c) Finally, the cancers produced in mice and in male rats by the estrogenic substances synthesized by Dodds and his co-workers, such as triphenylethylene (Robson and Bonser) and diethylstilboestrol (Lacassagne, 42, and Geschickter), have seriously impaired the theory correlating the carcinogenic action with the molecular structure of the hormone (or one of its derivatives). As these compounds only remotely resemble the sterols, it seems difficult to admit of their transformation either into a carcinogenic substance similar to methylcholanthrene or a substance related to the natural hormones. Nevertheless, it is quite admissible to suppose that the estrogenic and carcinogenic properties may be due to the presence of an identical functional radicle.

PARTICIPATION OF THE PITUITARY IN THE PRODUCTION OF MAMMARY CANCER BY ESTROGENIC SUBSTANCES

While the investigations mentioned above were being carried out, bit by bit, at first with contradictory results and later with more and more convincing observations, physiologists established the fact that the anterior pituitary hormone is indispensable to the development of the mammary gland and that estrin is not able alone to produce proliferation of the mammary ducts. These observations, which are capable of changing our whole outlook on the problem of hormonal action in the pathogenesis of mammary carcinoma, may be briefly reviewed.

(1) Rôle of the Pituitary in the Development of the Mammary Gland: Corner, in 1930, suggested a possible action of ovarian hormones on the mammary gland through the intermediary of the pituitary, after having obtained, in ovariectomized female rabbits, the development of glandular lobules and lacteal secretion by injections of anterior pituitary extracts. This observation has long remained open to discussion. Ruinen, Asdell and Seidenstein, Nelson (68) and others found that hypophysectomy did not prevent mammary gland development in rats, rabbits, or guinea-pigs receiving estrin injections. But opposite results were obtained, by Selye, Collip and Thomson in the rat, by Lyons and Pencharz and by Reece, Turner and Hill in the guinea-pig, and by Gomez, Turner, Gardner and Hill in the mouse, probably as a result of an improved operative technic which permitted more complete removal of the hypophysis. Despite injections of estrogenic substances administered for periods up to forty-five days after hypophysectomy, the mammary glands in all these animals were atrophied. Lacassagne and Chamorro observed the same phenomenon in mice injected with estrone for a period of five months after hypophysectomy. They also hypophysectomized mice which, after being subjected to weekly injections of 50 γ of estrone benzoate for three months, had developed a marked cystic hyperplasia of the mammary glands; though the hormonal injections were continued after the removal of
the pituitary, the mammary glands underwent rapid involution and were found to be completely atrophic after three or four weeks.

Gomez, Turner and Reece showed that the development of the mammary gland is not the result of a synergistic action of the pituitary and ovarian hormones, but suggested that the action of folliculin takes place through the pituitary gland. They found that transplantation to hypophysectomized guinea-pigs of the pituitary gland of rats previously treated with repeated injections of estrin, produced an increase in the size of the mammary gland, while the transplantation of the pituitary of a normal rat had no effect. Finally, Lewis, Turner and Gomez established the presence of a "mammogenic substance" in anterior pituitary extracts of pregnant sheep and cows, which, when injected into normal or castrated male mice, increased the growth of the mammary gland.

In view of these observations we can appreciate the possibility of determining the influence of estrogenic substances on the production of mammary carcinoma, by discovering whether this can occur in castrated female or male mice in which mammary hyperplasia has been brought about by pituitary extracts alone. Since, however, we cannot profitably discuss results of experiments not yet undertaken, we may better return to the basis of well established facts and examine the alterations produced by estrin in the pituitary, by which abnormal growth of the mammary gland could be indirectly produced.

(2) Action of Estrin on the Anterior Pituitary Lobe and Its Possible Rôle in the Production of Cancer: (a) Smith and Engle, in 1929, noted a decrease in the gonad-stimulating power of the pituitary of the guinea-pig during estrus, and Charipper and Haterius, in 1930, observed a reduction in the number of acidophilic cells during the same period in the rat. Proof of the inhibiting action of estrin on the pituitary secretion has been given by Meyer, Leonard, Hisaw and Martin, who found that the implantation in immature rats of the pituitary glands of animals treated with estrone injections markedly delays the opening of the vagina as compared with the implantation of pituitaries of control animals. Later Leonard, Meyer and Hisaw noticed that prolonged administration of estrin has the effect of increasing the size and weight of the pituitary, which takes on the aspect of a gland in physiological hyperactivity.

The considerable hypertrophy of the anterior pituitary under the influence of repeated injections of estrogenic substances, and the consecutive modifications in its histological structure, so well described by Halpern and d'Amour, have been confirmed by numerous authors (Nelson, 67; Desclin; Wolfe; Chadwick; Severinghaus). These consist essentially in the reduction of the number of basophil cells and in the "degranulated" aspect of the acidophil cells, whose number also decreases progressively while that of the chromophobes increases. Simultaneously there are signs that can be interpreted as indicating a great secretory activity, as congestion, increase in the amount of colloid, hypertrophy of the chondrioma and of the Golgi apparatus, etc. If the treatment is sufficiently prolonged the pituitary may undergo a real transformation into a hemorrhagic chromophobe adenoma (Cramer and Horning, 17).

(b) Having noticed, after prolonged treatment with estrin, the frequent co-existence of these pituitary changes and mammary cancer in mice belonging to a strain highly susceptible to cancer, Cramer and Horning (18) were able to
establish a relationship between the two phenomena. They found that mice of a strain predisposed to carcinoma might show a particular susceptibility to the general systemic effects of estrin. In such animals the pituitary responds to the introduction of a large amount of that hormone by a hypersecretion on the part of the acidophil cells, eventually reaching the point of "degranulation" of these elements and their extinction; finally a syndrome of hypopituitarism sets in, similar to that produced by destruction or removal of the gland. The cancer of the mammary gland is the result of this hormonal imbalance.

The pituitary hormones act as antagonists of the carcinogenic effect of estrin. In male mice which had received alternate injections of thyrotropic hormone and estrin for a period of seven months, Cramer and Horning (19) demonstrated microscopically the absence of the pituitary changes and mammary hyperplasia which develop following injections of estrin alone. In females of a high mammary cancer strain, receiving repeated injections of thyrotropic hormones, beginning at the age of two months, no case of cancer developed, though the animals were still alive at ages varying from ten to sixteen months. The adrenals, on the other hand, are endowed, according to these authors (20), with hormonal functions synergistic with those of the ovaries. It is thus possible by adrenalectomy to prevent or delay the appearance of mammary cancer in predisposed mice.

(c) Numerous objections have been raised to this theory. After treatment with equiline, or more especially with equilenine, the effects of which are less severe than those of estrone, mammary carcinomas may develop in animals in which there is apparently no histologic modification of the anterior pituitary lobe (39). If large doses of estrogenic substances suppress pituitary secretion, mammary involution and not hypertrophy should be the result, as is shown by the hypophysectomy experiments described above. The changes in the anterior pituitary lobe secondary to the administration of large doses of estrone are very different in various strains of mice, but no relation exists between the gravity of these alterations and susceptibility to cancer, since the most pronounced changes have been found in the pituitaries of strains totally free of mammary carcinoma (46). Asdell and Seidenstein found that injections of thyrotropic hormone, at a rate of 12.5 guinea-pig units weekly in young animals of a strain subject to cancer, did not counterbalance the effect on the mammary gland of a weekly injection of 50 γ of estrone benzoate; and treatment with the same quantity of thyrotropic hormone, begun in females after the age of sexual maturity, failed to prevent the occurrence of a mammary carcinoma in the usual period of time (Lacassagne, 43).

(d) Experiments recently published by Loeb and Kirtz lead to conclusions which are also contradictory to the conception of Cramer and Horning. These authors implanted under the skin of mice less than two months old, pituitaries from their sisters or brothers. The transplants, under these conditions, still survive after eight or ten months. In virgin females such transplants produce a marked hypertrophy and secretory activity of the mammary glands, and in animals of strains subject to cancer an increase in the percentage of mammary carcinomas as compared to virgin controls belonging to the same strains. On the other hand, no effect of pituitary transplantation was observed in castrated

---

Not 500 γ as was incorrectly stated in the original paper.
females or in males whose mammary glands remain rudimentary. The pituitary thus appears to act in cooperation with the ovaries. The authors believe that the activity of the corpora lutea may be a contributing factor determining the effectiveness of the transplanted pituitaries in their action on the mammary gland and, consequently, in the cancerization of that organ.

**ATTEMPTS AT HORMONAL PREVENTION OF MAMMARY CARCINOMA**

We shall exclude consideration of experiments with progesterone in combination with estrin, since it has produced no results, in the doses used, either in the form of an increase in the percentage of adenocarcinoma or as an antagonist of the carcinogenic action of the latter hormone (40).

(1) *Inhibition of Mammary Gland Development in Immature Female Mice by Testosterone* (Lacassagne and Raynaud, 48): Young female mice of a strain highly susceptible to cancer received biweekly injections of 2 mg. of testosterone propionate, beginning a few days after birth and continued until the animals died or were killed. Among 12 of these females which survived more than six months (of which 6 lived from one year to eighteen months), none developed mammary carcinoma and histologic examination showed an almost complete lack of development of the mammary glands, which retained grossly the appearance and morphology observed in the new-born female or in the adult male mouse.

In view of this result, two questions arise: What is the mechanism of this inhibiting action of testosterone on the development of the mammary gland in the mouse? Could a similar inhibiting action occur in adult female mice, to prevent the appearance of the carcinoma to which they are subject by heredity.

(2) *Possible Mechanism of Inhibition:* The physiological action of androgenous substances, which has been the subject of extended research in the past three years, is still obscure in many points, because of the different effects exerted by these substances among themselves and the dependence of these effects on the dose, technic of administration, and age and species of the experimental animal. Under these conditions, a discussion on the mechanism by which repeated injections of testosterone prevent the occurrence of mammary cancer can be undertaken only with great caution.

To the theory of an antagonism between the genital glands held by Steinach and Kun as a result of their experiments on the transplantation of these organs from one sex to the other, Moore and Price have for more than ten years opposed their hypothesis of an inhibition of the pituitary by the internal secretions of the sex glands, making available to the organism a reduced amount of gonadal-stimulating secretion, which would explain the apparently contradictory effects exerted by the male and female hormone on the same organ. It would seem, from numerous experiments undertaken during recent years, that both mechanisms play a part. Certain organs undergo a reversible transformation of structure under the alternating influence exerted directly on them by the male and female hormones, each of these counter-balancing the action of the other within the receptor organs. This holds true for the coagulating glands of the male (de Jongh, Korenchevsky, etc.), the vaginal epithelium (Courrier and Cohen-Salal and others), and the pituitary itself (Wolfe and Hamilton and others). On the contrary, the reaction of
certain other organs to the sexual hormones is, according to Moore and Price, principally by way of the pituitary. This applies to the gonads and to the mammary glands, as has been seen in the preceding section.

The inhibiting action of testosterone on the occurrence of mammary carcinoma in mice subjected to treatment before the establishment of effective functioning may be traced, therefore, through the following stages: inhibition of pituitary function, failure to produce gonadotropic substance, lack of secretion of folliculin, arrested development of the mammary gland, the prevention of mammary carcinoma being the direct consequence either of the last or the penultimate of these stages.

In the experiments of Lacassagne and Raynaud (47) microscopic examination of the ovaries, the mammary glands, and the pituitary of animals treated with testosterone propionate yielded the following results, consistent with the mechanism outlined above.

(a) In the ovaries the corpora lutea are absent, with atrophy of the interstitial gland and early atresia of the follicles, which degenerate before having reached the state of cavitation. These observations are similar to those made by others, i.e., that injections of testicular extracts or of testosterone suppress the estrus cycle (Lendle; Ihrke and D'Amour, etc.) and prevent follicular maturation (Zuckerman; Cotte, Martin, and Mankiewicz).

(b) The mammary glands are reduced to primary ducts only slightly branched and with no acinus formation. Histologically, only a few ducts are seen, with flattened lumen lined by a single layer of low and completely resting cells, which is practically the picture of the mammary gland of the adult male mouse. This observation confirms the idea that mammary carcinoma develops only in a gland which has previously undergone a certain degree of development. The results of the treatment of young immature mice by large doses of testosterone may thus be considered as a physiological castration leading to the prevention of mammary carcinoma by the same mechanism as ovariectomy performed before sexual maturity.

(c) The pituitary shows an anterior lobe rather reduced in size and containing a very large number of small acidophilic cells. These observations agree with those of most investigators who have studied the action of male hormones on the pituitary gland and who have shown that, like estrin, these substances prevent (Hohlweg and Dohrn) the histologic changes produced in the anterior lobe by castration (Nukariya, Lehmann, etc.); that the injection of testosterone propionate does not modify or only slightly diminishes the weight of the pituitary; that the basophil cells lose their granulations but the acidophil cells undergo no change (Wolfe and Hamilton, etc.).

It is not a matter then of an identical inhibiting effect by the female and male hormones on the pituitary gland, but rather of their antagonistic effect on the gland. In fact, it has just been shown that the changes in the anterior pituitary produced by estrin and those due to testosterone present certain opposite characteristics; in other words, the male hormone prevents the female hormone from exerting its influence on the pituitary (Wolfe and Hamilton, etc.). Despite the fact that the anterior pituitary of animals treated with estrin has rather the appearance of a hyperfunctioning gland and that of ani-
mals treated with testosterone gives the impression of being at rest, it seems
that according as one hormone or the other is in excess, this or that secretion
is either inhibited or increased. Zondek admits a similar dissociation in pit-
uitary secretions under the influence of estrin. In the present state of our
knowledge, it is permissible to suppose that in mice, estrin would act chiefly by
increasing the secretion of "mammogenic substance," and testosterone mainly
by inhibiting the secretion of gonadotropic substance. This would explain the
opposite effect of the two sexual hormones on the development of the mam-
mary gland and, in animals of strains subject to carcinoma, on the production
or prevention of mammary cancer.

(3) Experiments on the Prevention of Carcinoma in Adult Mice: The re-
sults obtained with testosterone, when used prior to the development of the
mammary gland, can be duplicated only imperfectly in adult animals submit-
ted to this treatment, since their mammary glands have already attained a
certain stage of maturity.

(a) Nevertheless, Nathanson and Andervont believe that they have suc-
cceeded, by this procedure, in preventing the occurrence of mammary carci-
noma in mice which have been pregnant once. Their experiment was carried
out on animals belonging to a strain in which 95 per cent of the females usually
develop cancer. Mice four and one-half months old, all of which had previ-
ously borne a single litter sacrificed after twenty-four hours, received 3 injec-
tions of 0.5 mg. of testosterone propionate weekly. While 20 female controls
showed mammary cancer in the usual period of time, i.e. around the eleventh
month, only 6 of the 20 treated animals had cancer. The tumors of these 6
animals remained single; they appeared early (at eight and one-half months),
had the usual course of two and one-half months' duration, and caused the
death of the animals at eleven months. The rate of cancerization was thus
reduced to 30 per cent. Even so, the authors estimate that in these 6 mice,
cancer probably existed microscopically at the very start of the treatment, and
continued to develop, since testosterone does not change the course of an
established cancer.

This last hypothesis seems rather difficult of acceptance. Mammary carci-
noma, once established, has a rapid course, and nothing justifies the belief that
it might remain unnoticed for four months, and that the total duration might
be six and one-half months, as would be the case in these experiments. Also,
it would be surprising for a cancer which usually appears around the eleventh
month to have arisen before four and one-half months in the strain used, as
such precocity is observed only in mice treated with large doses of estrogenic
substances.

(b) The possibility cannot be excluded that the male hormone, by early
restraint or inhibition of the ovarian secretion of young adult females, might
decrease the incidence of mammary cancer. This observation would be a
corollary of the experiment by Loeb, who showed that ovarian castration pre-
vents the production of this carcinoma when it is performed about the third or
fourth month, greatly diminishes the percentage when performed between the
fifth and seventh month, but is without effect if performed after the seventh
month. It has also been shown (Lacassagne) that if weekly injection of
estrone in male mice is discontinued after three months cancers may still oc-
cur, but less early and less frequently than in males continuously treated with
the estrogenic hormone.

The following experiment does not confirm the idea that treatment with
testosterone diminishes in any appreciable degree the occurrence of mammary
carcinoma in adult females, that is to say in mammary glands already subjected
to the influence of folliculin.

Six females of the R III strain, varying in age from four and one-half to
nine and one-half months, received 2 mg. of testosterone propionate in 2 in-
jections weekly. Only one of these females had had a litter; she had a carc-
noma 28 days after the beginning of treatment, when eight months old (it can
be admitted in this case that cancerization existed when the injections were
begun). The other five mice had been kept apart from males up to the be-
inning of the treatment. Two died without cancer, two and two and one-half
months after treatment, aged six and one-half and eight and one-half months.
A mammary carcinoma occurred in 3 animals, after five, six, and six and one-
half months of treatment, at the ages of ten, fifteen, and sixteen months. The
proportion of cancers and their periods of occurrence in these six females were
thus of the same order as in the non-treated females of R III strain.

CONCLUSIONS

This review of numerous experimental observations brought forth in recent
years by physiologists and oncologists shows the rapid evolution of the prob-
lem of the rôle of the hormones in the production of mammary carcinoma.
Despite these advances, the factor causing cancer remains uncertain. The
present state of the problem may be summarized as follows:

(a) Carcinoma can occur only in a mammary gland which has undergone
a certain degree of development.

(b) The development of the mammary gland is dependent upon the estro-
genic and pituitary hormones.

(c) But whether these stimulating factors play a direct rôle in the can-
cerization process, or only produce the anatomical development of the mam-
mary gland sufficient to allow the cancer process to manifest itself is still un-
determined.

(d) It is of interest to compare this evolution of knowledge relative to one
of the factors indispensable to the production of cancer (the hormone) to work
carried on at the same time by geneticists, such as Murray, Little, and Bittner,
on the second indispensable factor, namely "hereditary predisposition."

BIBLIOGRAPHY