The Occurrence of Benign and Malignant Mammary Lesions in Rats Treated with Crystalline Estrogen*

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The relationship of ovarian hormones to the development of the breast and, logically, to mammary cancer appeared clearly outlined in the results of early castration experiments on mice. Puberty ovariectomy inhibited normal growth of the breast, with the consequence that females of strains with mammary tissue highly susceptible by heredity to spontaneous malignant alteration failed to develop this lesion. Nevertheless, later experiments indicated that this generalization needs modification, for Woolley, Fekete, and Little (48) demonstrated that the adrenal gland is capable of assuming the function of the ovary in animals spayed within 24 hours after birth.

Estrogens in mice.—With the demonstration that mammary cancer can be induced in animals by various estrogenic preparations, a firm foundation seemed assured for the theory that cancer of the breast is a local manifestation of excessive stimulation of this organ by one of the substances responsible for its physiologic control, or of an abnormal stimulation determined by some endocrine dyscrasia. Proof for these hypotheses has been sought for preeminently in mice. It quickly became apparent from the observations of Lacassagne (39), Gardner and his coworkers (18), Burrows (7), Cramer and Horning (9), Suntzeff and his associates (43), Bonser, Stickland, and Connal (6), Twombly (46), Taylor and Waltmarf (45), and others that treatment of mice with estrogens gave rise to a higher percentage of mammary cancers than would occur normally, and at an earlier age in females of stocks genetically susceptible to the disease in a greater or lesser degree. Males of these same strains, which if untreated did not have tumors of the mamma, also developed cancer if they received estrogen. In the absence of the hereditary factor, however, the female sex hormone induced no mammary cancers even in females. It is worthy of note that exceptions to the former sequence have been contributed by Lacassagne (30), Cramer (8), Gardner (17), Allen (1), and Loeb (31).

Estrogens in rats.—Species other than the mouse have been less widely employed to elucidate the hormonal mechanisms related to mammary cancer. The rat, rarely the host of spontaneous malignant tumors of the mamma, also proved resistant to excessive estrogen stimulation in the experiments of Heiman and Krebsiel (28), Emge (14), Druckrey (10), Silvestroni (39), and Grumbruch (26). McEuen (32), on the other hand, observed an occasional cancer of the mamma, among other neoplasms, in rats given estrogen and painted with tar. Mammary cancer developed more readily in animals that received implants of pellets of crystalline hormone, as would be concluded from the publications of Geschickter (21, 22), and Noble, McEuen, and Collip (36). Some of the tumor-like growths described by the latter investigators appeared not to have progressed beyond the stage of hyperplasia, since they lacked the quality of autonomy. Thus regressions occurred following extraction of the estrogen pellet as well as after the administration of progesterone (35).

Dependence of action of estrogens on milk factor.—The view that excessive or abnormal estrogenic action, instead of acting directly as a carcinogenic stimulus, may only increase the total mammary tissue or induce chronic mammary hyperplasias which in turn are likely to be susceptible to other agents primarily responsible for the malignant alteration, finds some justification in observations on the factor transmitted with the milk. Twombly (47) was able to produce mammary cancer in C57 black mice—a strain not susceptible to this disease either spontaneously or if treated with massive doses of estrogens—by nursing estrogenized males on females of the R III strain, which has a high frequency of spontaneous mammary carcinoma. Bittner (4), on the other hand, inhibited tumor development in estrogenized A mice—a strain in which the females are normally susceptible to spontaneous mammary cancer—through fostering by C57 black mice. These results were confirmed by Shimkin and Andervont (38). From these observations, therefore, the importance of estrogenic stimulation appeared subordinate to, or possibly conditioned by, a factor transmitted in milk.

Benign and malignant mammary lesions produced by carcinogenic hydrocarbons.—While endocrine factors indubitably are of considerable importance for the induction of mammary neoplasms, observations with the carcinogenic hydrocarbons prove that they are not indispensable in the initiation of either benign or malignant lesions of the breast. Statistical evaluation of tumor production in animals by the hydrocarbons offers additional support for the contention that the malignant change occurs as the result of factors operating locally, and that the type of tumor induced depends upon the production of a permanent modification in a given cell after chance contact with a specific irritant. The significance of the mammary alterations induced by the hydrocarbons becomes readily apparent in the light of these concepts. Thus Stewart (40) noted the local development of mammary cancer about methylcholanthrene in C57H mice, a strain that is highly susceptible to mammary cancer, while Strong and Smith (41), Strong and Williams (42), and Bonser and Orr (5) reported a similar effect in mice genetically resistant to the spontaneous occurrence of mammary neoplasms. Estrogens, nevertheless, appear to exercise some determining influence on the site of action of absorbed hydrocarbons, for Perry and Ginzton (37) observed a considerable number of cancers of the breast and uterus in mice given the hormone and painted on the skin with dibenzanthracene.

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In rats and mice of known lineage, Dunning, Curtis, and Bullock (11) obtained a small number of mammary carcinomas about subcutaneous nodules of benzpyrene in paraffin. Later Dunning, Curtis, and Eisen (12) reported that methylcholanthrene possessed an even greater faculty of producing proliferative lesions in the breast of the rat. Proliferation of the columnar cells lining the ducts, squamous cell metaplasia of the hyperplastic ducts—transformed in a number of instances by continued function of the altered cells into squamous cell cysts—were found in males and females about focus of the carcinojen before and after the appearance of sarcoma. A small but not negligible number of animals developed mammary adenocarcinomas as a result of progressive action locally of the specific irritant without the intermediary squamous cell metaplasia, while in others squamous cell cancer developed from the metaplastic ducts or cysts.

Relation of absorbed hydrocarbons to mammary cancer.—A number of experiments, such as those of Maisin and Coolen (33), Mider and Morton (44), and Engelbreth-Holm (15), indicate that methylcholanthrene absorbed from a solution in benzol applied to the skin, especially if different areas are used successively to avoid cutaneous carcinogenesis, may assist the development of mammary cancer in genetically susceptible mice. The resulting increase in the number of mammary tumors, the incidence is significantly higher than in comparable experiments on the testes, the average time required for the absorption of estrogen occurred slowly and continuously (13). In performing the subcutaneous implantations no deliberate attempt was made to place the material in the mamma. As judged by the vaginal smear, or the position and functional state of the testes, the average time required for the absorption of estrogen was 157 ± 5.9 days. A minimal foreign body reaction developed about the nodules. They eventually became encysted in a relatively acellular connective tissue which in no instance showed evidence of sarcomatous transformation. With the exception of those animals that received a single dose of 1.0 mgm. and no further treatment, all were given supplementary quantities of 2.5 or 5.0 mgm. when the vaginal smear or condition of the testes indicated cessation of absorption from the earlier implanted foci. The latter rats therefore remained under the constant influence of exogenous estrogen during their entire lives. For this study 134 animals were used, of which 63 were females and 71 males.

The rats employed were members of the inbred Sherman strain, with the exception of a small number of animals of several unrelated inbred families. Spontaneous mammary lesions resembling those occurring in rats that received female sex hormone have not been observed in untreated rats. While fibroadenoma of the mamma has arisen occasionally in old, untreated Sherman females, a sufficient number of such neoplasms has not been collected to ascertain whether the incidence is significantly higher than in comparable members of other strains. Certainly the equal susceptibility of both sexes to exogenous estrogen can be accepted as corroborative evidence of the dependence of the mammary alterations on estrogenization, for spontaneous changes in the breast are practically non-existent in the male. The animals were maintained on the standard laboratory ration of equal parts Purina dog chow and Rockland rat diet with water as desired, supplemented by a liberal supply of fresh carrots and 6 drops of cod liver oil once weekly, a regime which has proved adequate for the growth and reproduction of normal rats.

The hormone employed, estradiol dipropionate, was administered to animals from 3 to 5 weeks of age. An early technic—subcutaneous implantation of weighed amounts of crystalline material through a small skin incision—employed in a few animals only, was superseded by the injection of the hormone dissolved in liquefied paraffin (melting point 48°C.) in a concentration of 5.0 mgm. per 0.1 cc. The warm paraffin, injected subcutaneously, intramuscularly, or intraperitoneally, solidified as firm ellipsoids from which absorption of estrogen occurred slowly and continuously. In performing the subcutaneous implantations no deliberate attempt was made to place the material in the mammal. As judged by the vaginal smear, or the position and functional state of the testes, the average time required for the absorption of estrogen was 157 ± 5.9 days. A minimal foreign body reaction developed about the nodules. They eventually became encysted in a relatively acellular connective tissue which in no instance showed evidence of sarcomatous transformation. With the exception of those animals that received a single dose of 1.0 mgm. and no further treatment, all were given supplementary quantities of 2.5 or 5.0 mgm. when the vaginal smear or condition of the testes indicated cessation of absorption from the earlier implanted foci. The latter rats therefore remained under the constant influence of exogenous estrogen during their entire lives. For this study 134 animals were used, of which 63 were females and 71 males.

In Table I the rats are grouped according to the amount of estrogen administered, which varied from 1 to 20 mgm. in divided doses. Table II shows the number which died in each month following the initial implantation. Although considerable variation exists in the rate of utilization of identical amounts of estrogen in different rats, no consistent correlation was discerned between the rapidity of absorption and the extent of the associated lesions. Thus the length of time for continuous action of effective quantities of exogenous estrogen plays an important role in determining the nature of the induced alterations. Therefore all rats are grouped together regardless of the total divided dosage.

1 Estradiol dipropionate was generously furnished by Dr. R. MacBrayer, Ciba Pharmaceutical Products, Inc., Summit, N. J.
Mammary Lesions

As the object of these experiments was primarily the investigation of mammary alterations, these will be described in greatest detail. Mammary tissue representative of successive stages of constant influence by estrogen was secured by biopsy when not made available through the death of animals. The organ was equally influenced throughout, and the absence of more extensive local changes about hormone implanted in it by chance attested the generalized nature of the estrogenic influence. As the type of lesion encountered depended largely upon the duration of abnormal estrogenic action, definite architectural patterns constituted in general the outstanding morphologic characteristics of successive stages in a continuously developing alteration determined by a constantly available agent. Nevertheless, this division into stages proved somewhat arbitrary in that the presence of the dominant lesion corresponding with a certain interval did not exclude necessarily the possible discovery of areas characteristic of earlier or later reactions. The alterations, as they were continuous, evolved without clear demarcation through successive periods.

Proliferation of duct epithelium.—Although some details pertaining to the ultimate nature of the hormonal control of mammary growth and function have not yet been completely elucidated, many facts rest upon a firm experimental foundation. In addition to physical factors and pituitary stimuli the 2 hormones elaborated in the ovary, estrogen and progesterone, are of prime importance. While no claims can be advanced for a strict delimitation of their effects, estrogen functions primarily as a stimulator of duct development, and progesterone, when acting in conjunction with the former, as a motivator of acinar growth and possibly of function. Proof of these contentions is made readily available by a study of gross mounts of the breast and corroborative histologic preparations, as is known from numbers of reports in the literature and as can be adduced from the present series of animals.

A considerable degree of diffuse proliferation occurred in the breast as early as 26 days after the implantation of crystalline estrogen. Microscopically the prominent ducts were evident immediately, as a consequence of hyperplasia of the columnar lining cells. Instead of the 1 or 2 layers of resting cells normally found at this age, considerable areas of the ductal lumina were filled with proliferating cells, well confined, however, by the basement membrane. Mitotic figures, although common, presented no evidence of being atypical. Acinar development was minimal or absent (Fig. 1).

Secretion.—After approximately 40 to 60 days, when the primary proliferative stage had attained its zenith, signs of functional activity appeared in the duct epithelium. Instead of the profuse aggregation of elements characteristic of the earlier stage there was now an arrangement of the cells in several orderly layers. Secretory vacuoles appeared in the cytoplasm and eventually, after having been extruded or desquamated into the lumina together with cellular fragments, coalesced to form pale eosinophilic masses.

At the height of the diffuse secretory phase induced by crystalline estrogen, gross mounts offered striking proof of the generalized growth of the mamma, particularly evident in the ducts. The great increase in the number of branchings and in the caliber of the tubules contrasted strongly with the relatively undeveloped duct system of untreated control females and the rudimentary mammary elements of normal male rats.

Duct dilatation.—This logical development occurred at 60 to 90 days as a result of the continued activity of the secreting cells and retention of the collected secretions. For the function induced by an abnormal hormonal concentration, in the absence of other factors required for lactation, was necessarily abortive and the elaborated fluid, after stagnating, produced enlargement of the lumina.

Duct cystosis.—This more advanced development of the former stage, the formation of poorly defined but fairly well localized cystic zones visible macroscopically, at times 0.5 to almost 1.0 cm. in diameter and containing a thick fluid comparable in consistency with milk, was attained at 90 to 150 days. Flattening and desquamation of the lining cells accompanied large accumulations of cystic fluid, probably as a result of cellular disintegration consequent on mounting intracyctic pressure. The cystic zones displaced the fat cells normally present between mammary lobules.

DESCRIPTION OF FIGURES 1 TO 3

Fig. 1.—Female. Proliferative lesions of early stage in mammary ducts. Estradiol dipropionate, 3.0 mgm., 32 days. Mag. × 375.

Fig. 2.—Female. Stage of diffuse functional activity in mammary ducts, in which collected secretions produce dilatation and cystic zones. Note the almost complete displacement of the fat cells. Estradiol dipropionate, 6.0 mgm., 132 days. Mag. × 55.

Fig. 3.—Female. Fibrosis about enlarged ducts. Note the flattened, inactive epithelium lining the spaces, the inspissated secretions containing desquamated cells, beginning distortions of the ducts, reappearance of fat cells, and the epithelium of some ducts still in the stage of secretion. Estradiol dipropionate, 17.5 mgm., 387 days. Mag. × 157.5.
The progressive but overlapping alterations consisting of secretion leading to duct cystosis are illustrated in Fig. 2.

Fibrosis.—Stimulated growth of the periductal connective tissue, followed by increasing contractions and distortions of the previously enlarged ducts, became evident at 150 to 180 days. Varying intensities of these alterations persisted in animals that survived longer. Purely mechanical factors associated with outward pressure of the overdistended ducts, or chemical irritation resulting from dissipation of the secretions or products of their decomposition, possibly accounted for the fibrosis of progressively increasing severity.

The condition of the surviving epithelium, now apparently in a stage of relative passivity, appeared to be secondary to the development of the lesions in the surrounding stroma. The new connective tissue, at first sparse and cellular, became transformed later into hyalinized bundles. Physical alterations in the masses of connective tissue gave rise to bizarre distortions of the enlarged duct spaces, including intracystic intrusions, especially when viable proliferating or secreting cells persisted in the epithelial layers (Fig. 3).

The fibrosis eventually became so diffuse as to displace largely the fatty tissue which had reappeared after the initial development of contraction of the enlarged or cystic ducts. These now persisted as more isolated, irregularly shaped spaces while in some instances their dimensions were reduced further to that of shrunken but elongated tubes. The remaining epithelial cells, usually arranged in single layers, gave the impression of having undergone extensive regressive changes. Nevertheless, in some areas they appeared indistinguishable from normal resting cells. Foci of round cells and macrophages were encountered not uncommonly in the stroma.

The more advanced condition (Fig. 4) and the well developed fibrotic mammary lesion (Fig. 5) do not differ significantly from certain forms of chronic cystic mastitis observed in the human breast. In some instances the fibrosing process (Fig. 6) may eventually give rise to a more intimate type of sclerosing adenomatosis by producing dissociation of individual mammary tubules. This likewise is the counterpart of a chronic benign mammary proliferation occurring in the human subject.

While on the whole the different stages appeared to follow in sequence as part of a continuous process, it is necessary to emphasize that not all animals maintained for comparable periods under the constant influence of slowly absorbed estrogen showed identically advanced lesions, nor were different areas of the mamma of a single animal equally affected.

Late secondary epithelial proliferation.—Lesions of this type were observed in 6 animals, dead or sacrificed after 242 or more days. Unquestionably some stage of the chronic fibrotic lesion with persisting although altered ducts and irregular cystic formations constituted the outstanding mammary alteration found in these rats. In addition, irregularly distributed in these lesions were found foci, in some cases multiple, of epithelial cells again presenting evidence of proliferative vigor. As they were arranged in several layers they caused either partial or complete obliteration of the lumina into which they grew. They were identified readily by the deeper stain invariably assumed by their nuclei. The formation of secondary glands in the solid epithelial masses was common, and mitotic figures were encountered frequently. Nevertheless the cells showed no evidence of the disorder characteristic of neoplasia, and invasion of the surrounding stroma did not occur. Localized zones of papilliferous proliferation of larger, somewhat cosinophilic cells have been observed occasionally, but in no instance could these cells be compared directly with the so called pale epithelium of human chronic mastitis.

Fig. 7 is a longitudinal view of a large, originally cystic duct with imposing growth of the lining cells. In Fig. 8 this type of proliferation is illustrated in a number of ducts.

Malignant alteration.—Lesions, interpreted as carcinoma of the breast because of the massiveness of the process, its invasive qualities, and the attendant histologic signs commonly accepted as denoting malignant disease, were found in 2 rats. In one the zone of neoplastic change was discovered microscopically in an animal with a chronic fibrotic mammary lesion, dead 376 days after the first implantation of estrogen. The growth (Fig. 9) is a well differentiated adenocarcinoma which appears to have originated in an area characteristic of the earlier chronic benign lesion.

DESCRIPTION OF FIGURES 4 TO 6

Fig. 4.—Female. More extensive fibrosis about a large cyst, with many irregularly shaped contracted ducts in the abundant stroma. The tissue is from the same animal as that illustrated in Fig. 5. Mag. × 90.

Fig. 5.—Male. Advanced fibrosis of the mammary tissue. Note the extensively developed fibrous tissue containing remnants of enlarged, distorted ducts and contracted epithelial spaces, and the displacement of the fat. Estradiol dipropionate, 10.0 mgm., 181 days. Mag. × 35.

Fig. 6.—Female. Sclerosing adenomatosis of the breast produced by advanced fibrosis dissociating individual, contracted mammary tubules. Estradiol dipropionate, 12.5 mgm., 551 days. Mag. × 200.
The second animal with cancer, sacrificed after 803 days, had 4 circumscribed subcutaneous masses 2 to 3 cm. in diameter. The mamma was diffusely involved by benign fibrocystic disease. Histologically the neoplasms were adenocarcinomas with minimal variation in the architecture of individual growths (Fig. 10). Gross metastases were not apparent. Transplantations were not attempted.

Changes in Other Tissues

General influence.—The generalized effect of the constantly absorbed estrogen was apparent immediately from the diminished size of the animals, but in the absence of complications or secondary infections their general health did not appear seriously impaired.

Genitalia.—Ovarian function remained in abeyance, the follicles persisting without evidence of further development or the formation of corpora lutea while...

DESCRIPTION OF FIGURES 8 TO 11

Fig. 8.—Female. Multiple foci of late secondary epithelial proliferation in mammary ducts. Note the frequent mitoses, some atypical cells, but the absence of the disorder of cancer. Estradiol dipropionate, 11.5 mgm., 333 days. Mag. X 200.

Fig. 9.—Female. Mammary adenocarcinoma situated in an area of chronic fibrocystic disease. Estradiol dipropionate, 12.5 mgm., 376 days. Mag. X 200.

Fig. 10.—Female. Mammary adenocarcinoma. Estradiol dipropionate, 1.0 mgm., 803 days. Mag. X 375.

Fig. 11.—Squamous cell cancer of the cervix. This tumor developed in the same animal as the mammary lesion illustrated in Fig. 6. Mag. X 200.
Figs. 8-11
implanted estrogen continued available. Cystic changes were common in animals surviving 1 year or longer.

Initially some signs of proliferation were manifest in the cylindrical epithelium of the uterine horns. Squamous cell metaplasia followed in many instances, while in a considerable number pyometra developed as a final complication. It was surprising that animals with such enormous but encapsulated masses of purulent material survived for many months. In the absence of infection, hyalinization developed, at first in the submucosa, later in the entire uterine wall.

In the cervix benign proliferation of the squamous cell zone was an invariable finding. One animal, killed after 551 days, had a squamous cell cancer of the cervix (Fig. 11). No vaginal tumors were found.

In the male the undescended, stunted testes remained functionally in a rudimentary state. The small seminiferous tubules were lined by 2 or 3 layers of cells which showed no sign of spermatogenesis. Evidently these alterations were reversible, for normal development eventually took place in estrogenized animals if, following the complete utilization of a supply of estrogen, the exogenous hormone was not replaced (13). In the seminal vesicles, flattening of the nonfunctioning epithelium accompanied by fibrosis of the wall of the organ appeared in all animals.

Adrenal gland.—Prominent changes were not found. Small cortical cysts appeared in some rats after 1 year.

Hypophysis.—Hyperplasia of the anterior lobe has been described consistently in estrogenized animals. In the present series it was evident that the time of appearance of this change was delayed as a result of slow absorption of the responsible hormone. Rats surviving 150 days or longer showed some hypophysial enlargement, but the degree appeared unrelated to the lesions in other tissues.

Other organs.—Consistent evidence of change was not discernible in the thyroid, thymus, or pancreas.

Other Tumors

Lymphosarcoma.—Lymphosarcoma, originating in the mesenteric lymph nodes, was present in 2 rats, a male that received 1 mgm. of estrogen and died after 338 days and a female treated with 17.5 mgm. which succumbed after 359 days. As these growths have been observed in untreated animals of this strain their relationship to estrogen, if any, is difficult to evaluate.

DISCUSSION

From the results of these investigations it is readily apparent that slow constant absorption of an estrogen, estradiol dipropionate, from paraffin foci containing the crystalline material, produces in rats of known genetic constitution and of either sex a chronic proliferative mammary lesion which is preeminently benign. The induced alterations, which affect primarily the ducts, although continuous parts of a single basic process, pass imperceptibly and without uniformity in different animals or in various regions of the breast of one animal through the consecutive stages of proliferation, secretion, dilatation, and cystosis. These expressions of abnormal estrogenic activity, primarily affecting the epithelium, are succeeded by fibrotic changes in the supporting tissue which are initially periductal but which ultimately become more intense and finally involve large areas of the interepithelial tissues. Not without some justification the characteristic diffuse lesion found in many rats after 180 days or more may be considered an experimental counterpart of chronic cystic mastitis of the human subject.

The term "chronic cystic mastitis" is acknowledged by most investigators as unfortunate. Nevertheless, it continues to be employed to designate an ill defined group of benign mammary lesions although it is now universally recognized that inflammation plays no etiologic role. An endocrinologic basis for the condition suggests itself from many of its outstanding clinical aspects, such as: the occurrence in persons relatively young and still sexually active; the frequent and consistent variation in degree of the lesions, both objectively and symptomatically, with the menstrual cycle; the commonly associated menstrual disorders; the evidence of other abnormalities in the sexual apparatus or the history of earlier deviations from the normal; and the amelioration often produced by hormonal therapy. Although Taylor (44) finds no definite proof of an endocrine basis, he does admit that ovarian dysfunction cannot be entirely excluded in patients with the syndrome. In a more recent survey, containing an admirable account of earlier investigations, Taylor and Waltman (45) state that the long continued injection of mice with estrogen in oil, while eliciting adenomatous proliferation of the mamma in animals hereditarily susceptible to cancer and duct hyperplasia in other strains, fails to induce the fibrocystic lesion with complex epithelial proliferations that is so characteristic of human chronic mastitis. Geschickter (23), on the other hand, who bases his contention on urinary hormone assays, believes that a reduction in the elaboration of the corpus luteum hormone associated with hyperestrinism is responsible for the disease. In rats, according to this author, it can be reproduced by treatment with excess of estrogen combined with progesterone or testosterone. Grumbrecht (26), arguing from experimental evidence in the rat, unhesitatingly classifies chronic cystic mastitis as an endocrine abnormality.

In employing the rat with the object of simulating experimentally a human disorder as variable in its
manifestations and at the same time as extensive in scope as chronic cystic mastitis, it is important to realize the existence of biologic and physical differences in the normal mammary tissue of the species. While it is impossible to evaluate the significance of these differences, they must operate as factors conditioning the comparability of the spontaneous and induced forms of an endocrine disease. Taylor and Waltman (45) regard 2 alterations as characteristic of chronic mastitis: adenofibrosis and duct hyperplasia. Geschickter (23) prefers the terms mastodynia and adenosis for essentially the same manifestations. With justice the former state that, for an investigator to assert that the production of chronic mastitis has been achieved experimentally, "a fair proportion of the morphologic forms of the human disease must be demonstrable."

The late stages (Figs. 4 to 8) of the lesions in the mamma of the rats employed in the present experiment, produced by slow but constant absorption of estrogen, appear to imitate chronic cystic mastitis in the human subject closely enough to allow the supposition that one of the factors responsible for this disease is prolonged excessive or abnormal influence by the female sex hormone. The fibrosis is one of the cardinal late features of the experimental lesion, and to judge from a study of successive stages it is secondary to proliferation and function in the duct epithelium, and more especially to dilatation of the duct lumina by secretions. The subsequent distortions, contractions, and disorganization of persisting mammary ducts are physical complications occasioned by the stagnated secretions and this new abnormal mass of connective tissue, subject itself to hyalinization.

The late secondary epithelial proliferations, occurring in a few animals with fibrocystic mammary disease, form the logical counterpart of the duct proliferations that are such an important feature of the disorder in the human breast. In both species it is difficult to appraise the significance of this process. The cells appear unquestionably to be proliferating vigorously. However, the absence of genuinely atypical qualities confirms their essentially benign nature. Whether malignant change is the ultimate fate of cells of this type in subjects surviving for a sufficient time, is manifestly impossible to ascertain. The supposition appears tenable. These cells, rather than the remainder in an exhausted or resting stage, do give the appearance of renewed proliferation after the completion of a cycle of growth, function, and cessation of activity through the operation of a constant hormonal stimulus. The sequence is not unlike that observed by Greene (25) in certain types of spontaneous cystic mammary disease and cancers in the rabbit.

A question of paramount importance remains: the relationship of the female sex hormone to mammary cancer. Is there sufficient evidence for the assertion that the estrogens are capable of functioning as carcinogenic agents? In our present state of knowledge it is perhaps useless to probe too deeply into the concept "carcinogen." It is sufficient to recall the diversity of agents which may elicit neoplasia either occasionally or inevitably: physical factors such as ultraviolet light, radium, or roentgen radiations; many otherwise indifferent chemical substances which induce tumors only when present in abnormal concentration or at unusual sites, as sugars and acids; certain azo dyes which induce growths in the liver; cysticercus larvae, giving rise in the rat to sarcoma of the liver, the normal habitat of the parasite, but capable of causing tumors at other sites as well, as, for example, after they have been transported artificially to the subcutaneous tissue; and finally the carcinogenic hydrocarbons, the most potent of all, with a more generalized activity and surmised to act directly and locally on the cell to transform it irrevocably into a malignant subtype. The exact position of the estrogens in this series is debatable.

In the experiments recorded in this communication the late secondary epithelial proliferation, while essentially a benign manifestation of chronic fibrocystic mammary disease yet suggestive of possible subsequent malignant alteration, was observed in 6, and lesions interpreted as mammary cancer were found in 2 of 103 estrogenized rats that survived for 242 days, the minimal time at which these proliferative lesions were evident. Serial examination, instead of isolated sections from representative macroscopic zones, would perhaps have yielded a greater incidence, especially of the earlier type, but it is unlikely that the high incidence of cancer newly reported by Geschickter and Byrnes (24) in estrogenized rats would have been approached.

The incidence (8 per cent) of combined late proliferations and cancer of the breast in estrogenized rats, mentioned in the preceding paragraph, is not negligible, however, if compared to the virtual non-existence of these lesions spontaneously in the strains employed. On this assumption—the almost certain likelihood that the lesions will not occur in the absence of the treatment instituted—abnormal estrogen stimulation can be classified conditionally as one of the factors responsible for carcinoma of the breast. The relationship of the hormone to the multiple breast cancers first observed in one animal 803 days after the single administration of 1.0 mgm. crystalline material is hard to determine, for in an animal given this dose the exogenous estrogen must have disappeared some time prior to the inception of the tumor. The single instance of carcinoma of the cervix among
many examples of benign proliferations of this part in estrogized rats, while not comparable with the incidence of cervical cancer observed by Allen and Gardner (2) in mice given estrogens, is of interest, since a comparable tumor in untreated rats of the strain is as yet unrecorded. Although carcinomas occurred in rats that had received estrogen, there are, nevertheless, serious objections to admitting that estrogen, even though acting under artificial conditions, functions directly as a carcinogen:—

1. Slowly and constantly absorbed exogenous estrogen acts only on those tissues normally under the control of a physiologic supply of the endogenous hormone. True carcinogens possess a more universal scale of potency.

2. Tumors, either sarcoma or mammary carcinoma, are not produced locally at the point of maximal concentration of the crystalline hormone incorporated in paraffin, a vehicle inducing a mild, nonspecific, localized irritative effect. It may be added, however, that a few sarcomas have been recorded by Gardner and his associates (19) in mice at the site of injection of estrogen in oil.

3. The generalized mammary alterations produced by estrogen pass through a complete cycle of cellular proliferation, function, and exhaustion despite the constant presence of the original inciting agent. The malignancy of the early proliferations, considered by Geschickter (22) to be cancer, is manifestly disproved by the subsequent course of these lesions. It may be logical to assume, especially with the analogy of the local action of carcinogenic hydrocarbons in mind, that the late proliferations, leading ultimately in some cases to true neoplasia, are related to some superimposed local phenomena the nature of which cannot be surmised.

The mode of action of the carcinogenic hydrocarbons is strikingly different from that of the estrogens. The changes progress from an indifferent or benign reaction to frank malignant disease, and if a sufficient concentration is available for an adequate period practically all the treated animals develop malignant tumors.

An additional hypothesis, not susceptible of proof, has been suggested for the origin of cancer in estrogenized mammary glands. The hormone increases the total amount of breast tissue and also, therefore, the probability of chance occurrence of the late alterations, an event which would be equally liable to occur if the increase were wholly constituted by normal elements.

The mammary tissue of female mice of strains susceptible to spontaneous mammary cancer presents some differences from that found in resistant mice. Gardner, Strong, and Smith (20); Fekete (16); Taylor and Waltman (45); and many years earlier, though not in inbred strains, Haaland (27), all record persisting foci of adenomatous hyperplasia in susceptible strains prior to the development of tumors. These probably constitute the precocious morphologic criteria of a hereditary predisposition to malignant disease, manifested in a localized area of specific tissue. The administration of estrogen to such animals accentuates this development. Lesions like this do not arise under estrogen in mice of resistant strains, which instead show only cystic changes in secreting ducts. Lacsague (30) considers these two processes to be a manifestation of different inherited susceptibilities to the hormone.

The effects of estrogen on the rat, well known to be highly resistant to mammary cancer, include, in addition to the changes occurring in resistant mice, a tendency to secondary fibrosis of the supporting tissues. All this appears to indicate that the rat, on the whole, may logically be classified with the insusceptible group of mice.

SUMMARY

Crystalline estradiol dipropionate, implanted in rats of known genetic constitution and from 3 to 5 weeks of age, induced chronic diffuse alterations in the genitomammary system of both sexes, among which a fibrocystic mammary disorder, preeminently of a benign nature, was the outstanding manifestation. The lesions in the breast developed as a sequence of events characterized by considerable variability but occurring in all animals. The initial manifestations were present in the epithelium and consisted in proliferation, chiefly of the ducts; signs of functional activity by the cells; microscopic, followed by macroscopically visible enlargement of the lumina as a consequence of accumulated secretions; and exhaustion of cellular activity with subsequent evidence of disintegration of the cells or reversion of the epithelium to a resting stage. These lesions appeared responsible for the succeeding changes in the stroma. Proliferation of the fibrous tissue about cystic ducts was followed by diffuse fibrosis. This gave rise to contraction and distortion of the persisting mammary elements, and resulted ultimately in the production of a state comparable in many respects with human chronic cystic mastitis. With the later development of somewhat more atypical foci of secondary proliferation of the duct epithelium in the involved zones, this comparison appeared even more appropriate. Lesions interpreted as mammary adenocarcinoma occurred in 2 rats. Of associated changes in other tissues, a single example of squamous cell cancer of the cervix was of interest. The manifestations in animals that received estrogen have not been observed in untreated control rats.
While the etiologic agent which produces in the rat a chronic, benign, fibrocytic mammary lesion was estrogen, slowly but constantly absorbed from a focus containing the crystalline material, it is concluded that additional factors of an unknown nature, possibly acting locally, are more directly concerned with the origin of the late secondary epithelial proliferations and true neoplasia.

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