A COMPARATIVE MORPHOLOGICAL STUDY OF THE MAMMARY GLAND IN A HIGH AND A LOW TUMOR STRAIN OF MICE *

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In the Little-Murray inbred dilute brown strain of mice 80 per cent of the females with a normal reproductive history develop spontaneous carcinoma of the mammary gland. For this reason the strain can be considered as a high tumor line. In the C57 black strain of mice spontaneous mammary carcinoma appears in less than 1 per cent of the breeding females. This strain can, therefore, be considered as a low tumor line. The tumors are detected when they are large enough to be palpable. At this time microscopic examination usually reveals a well established malignant condition. By sectioning and comparing many mammary glands of the two strains it was thought that it might be possible to detect certain early structural changes and differences which might be considered as steps leading to abnormality. It is the purpose of this paper to describe the results of such an investigation.

The structure of the mammary gland of normal mice has been studied by several investigators. Among the more recent reports those of Turner and Gomez,[1] and Cole [2] are the most comprehensive.

Gibson [3] made a comparative study of the life history of the mammary glands of two strains of albino mice, one of which was a high, the other a low tumor strain. In the high tumor line she noted different anomalies in the nipple development, neoplasms developing in zones of chronic cystic mastitis, and the fact that the epithelial elements of the gland of the high tumor strain were inclined to metaplasia rather than to atrophy as age advances. Gardner and Strong [4] studied the mammary glands of virgin females in ten strains of mice differing in susceptibility to spontaneous neoplasms. They found no structural factor in the development of the mammary gland associated with the intrinsic hereditary predisposition to mammary carcinoma in mice.

The present investigation started with the examination of 1

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week old animals, continued at weekly intervals until the 4th week, and at biweekly intervals until the first pregnancy — about 6 to 8 weeks. From this time on the physiological condition of the mammary gland was taken as a basis for comparison rather than the chronological age of the animals, because it is important to know the condition of the females in regard to their reproductive phase at the time the mammary glands of two animals are compared. Without this knowledge the examination would lead to erroneous interpretation.

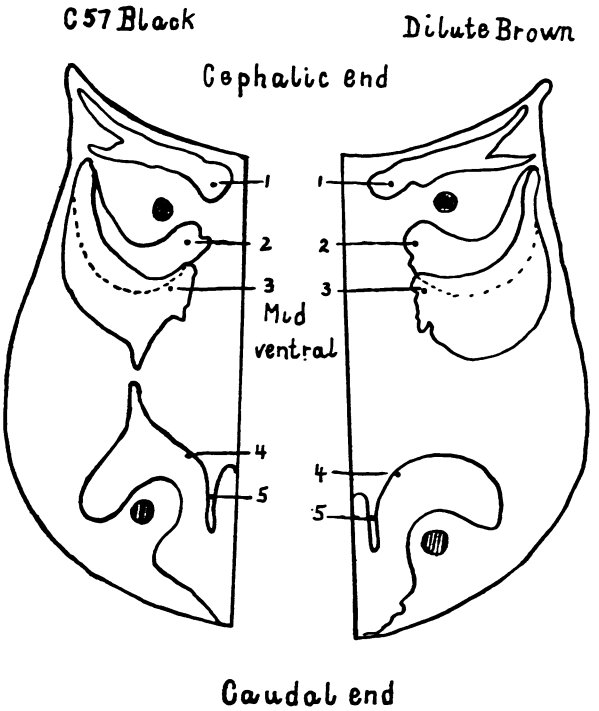
Pregnancy was timed from the appearance of the vaginal plug. As a routine the animals were separated from the breeding pen to individual pens at the later stages of their pregnancy. They were kept there until the young mice were weaned — 3 to 4 weeks after birth. The date of weaning depends somewhat on the size of the litter and general condition of the offspring. On the average a breeding female has a litter every 2 months.

Technical Procedure: The animals were killed by ether. The skin was cut open on the mid-dorsal line and carefully removed, with the subcutaneous fat pads in normal position. The skin was spread and pinned out on a layer of paraffin hardened on the bottom of a glass dish and flooded with the fixing fluid. A mixture of formaldehyde (10 cc.), 70 per cent alcohol (100 cc.) and acetic acid (5 cc.) was used for fixation. All the glands were carefully studied under a dissecting microscope. All the glands with the nipples from the right side were cut away from the skin surface, dehydrated, cleared and embedded in paraffin. In the earlier stages serial sections were cut at right angles to the skin surface. In the later stages the sections were cut parallel with the skin surface. This way many sections of full surface of the glands were available for examination. Hematoxylin and eosin stains were used.

Mice have 5 nipples on each side — 3 in the thoracic and 2 in the abdomino-inguinal region. Each nipple with its ducts, collaterals and terminal branches is a separate unit and is not in communication with the others. Throughout this work the glands will be designated with numbers from 1 to 5 starting with the one most cephalad.

It was observed that a difference exists between the extension of the fully developed glands of the dilute brown and black ani-

mals. The distance between the thoracic and inguinal glands in the dilute brown mice is greater than in the black mice. Text-figure 1 is an actual copy of the outlines of the glands of the two strains. While this greater distance was found to be constant in the dilute brown mice, in a few cases the extension of the gland in the black mice was similar to that in the dilute browns.



TEXT-FIG. 1. Diagram of the skins of the inner surface in the two strains of mice showing the position of the mammary glands. The drawing was made by tracing the outlines as they appeared on carefully skinned specimens. The position of the nipples is shown by numbered dots. The intermittent lines show the outlines of the 2nd glands which are overlapped by the 3rd glands. The shaded circles mark the position of the legs.

Observations: The nipples of the 1 week old mice can be located under a dissecting microscope. From each nipple one duct leads to the subcutaneous fat pad where it branches into a few secondary ducts. The subcutaneous fat pads are divided into lobes and lobules, and have a good blood supply. The main duct of the 1st

nipple grows cephalad, those of the 2nd, 3rd and 4th nipples grow dorsolaterally, and the main duct of the 5th nipple grows caudad. The primary duct of the 5th nipple has a longer, straight unbranched portion than any of the others.

Microscopically it is shown that at the site of the nipple the epithelium is thickened and the stratum granulosum and germinativum form a bell shaped epithelial cone which projects down into the subcutaneous tissue. In the middle of this the primary duct penetrates at a right angle to the skin surface, turning parallel with the skin surface as soon as it reaches the subcutaneous tissue. The lining of this duct is very compact and consists of low cuboidal epithelial cells. The epithelial cells of the secondary ducts are somewhat taller than those in the main duct. A fibrous envelope surrounding the ducts becomes gradually thinner toward the end distal to the nipple.

When the animals are 2 weeks old the skin at the site of the nipples is more elevated. The sections show that the bell shaped epithelial cone projects somewhat deeper and is surrounded by a sulcus. The ducts are longer and have more collateral branches.

At 3 weeks it is more difficult to detect the area of the nipples because the hair is growing rapidly and covers them. Microscopically sections show that the sulcus surrounding the nipples is deeper, and circular epithelial folds are beginning to form around the nipple. The ducts are considerably longer and have more side branches.

When the glands are fully developed the 2nd and 3rd glands overlap each other and are separated by a thin layer of muscle — the panniculus carnosus. The duct system of the 3rd gland has to penetrate through this muscle layer. This probably is the explanation of the fact that ducts of the 3rd nipple are somewhat slower than the others in development.

At 4 weeks the nipples are elevated above the skin surface and the circular folds are visible. The circular folds give the elasticity to the nipple which can be considerably stretched out by the young mice at the time of nursing. The development of the duct system has progressed further and produced more side branches.

The development progresses steadily up to the age of 6 weeks. This completes the prepubertal period which is characterized by regular progressive growth.

Between the ages of 6 and 8 weeks female mice reach sexual maturity. The changes that take place in the gland after that time are under the influence of the periodic sexual cycles of the animal. At the time of the first ovulation an acceleration of growth occurs and the ducts develop many side branches. The distal ends of some of the side branches terminate in bulb-like enlargements. Microscopically the end bulbs show that they are lined by many layers of cuboidal epithelial cells. Mitotic figures are numerous and signify rapid growth. The stroma around the end bulbs shows slightly increased fibrosis (Fig. 1).

A slight increase in the growth rate and dilatation of the ducts is noticeable every time the animal approaches estrus and is followed by a slight regression. If ovulation is followed by fertilization and pregnancy, development progresses rapidly. The ducts increase in length and many side branches are formed. The subcutaneous fat pads seem to limit the growth of the gland system and the original lobes and lobules of this adipose tissue form the framework of the fully developed gland. If fertilization does not occur there is a slight regression.

In order to be able to observe the possible individual variations within the normal, more than one animal of each of these stages was studied. The glands of 11 dilute brown and 11 C57 black mice were sectioned and examined up to this stage and showed no significant morphological difference.

First Pregnancy: During pregnancy the epithelial elements of the glands gradually enlarge by cell division. An increase in the number of mitotic figures is definitely noticeable at the 4th to 5th days of pregnancy and reaches its peak at about the 11th to 12th days (Fig. 2). This was ascertained by counting the mitotic figures in several cross sections of ducts. No great difference was found in the rate of growth between the dilute brown and the C57 black mice.

During this period many end bulbs are present at the distal ends of the ducts. After the first litter has been raised end bulbs do not occur on the glands during subsequent pregnancies. By further development all the bud-like endings and the terminal twigs dilate and unfold into alveoli.

At 14 to 15 days of pregnancy the glandular system is well developed and mitotic figures are rare. Only at the distal ends

away from the nipple are the ducts still growing. Further development consists of hypertrophy and an increase in size of the individual epithelial cells and the enlargement of the lumens. The ducts end in terminal alveoli which at this stage begin to show secretory activity. This activity starts first in the alveoli proximal to the nipple and progresses gradually distally. First small droplets, later larger vacuoles appear in the cytoplasm. The nucleus is pushed to the periphery away from the lumen. At 17 to 19 days secretory activity is generally well established throughout (Fig. 3). Parallel with the glandular elements there is an intensive development of blood vessels. The developing ducts follow the course of these vessels and capillaries are seen to be in intimate contact with the secreting epithelial cells. The developing glandular system occupies more and more space and the adipose tissue of the fat pads is rapidly diminishing.

The glands of 7 dilute brown and 6 black mice were included in this group. The sections were cut parallel with the skin surface, making the whole extent of the glands available for examination. All the mice were between the age of 2 and 3½ months old.

No structural difference was found between the two strains of mice.

From these observations it is evident that the changes taking place in the mammary gland during pregnancy can be divided into two periods. During the first period, which lasts until the 13th to 14th day of pregnancy, there is an acceleration in mitotic activity which results in a numerical increase of the epithelial cells of the glands. During the second period the epithelial cells increase in size and begin to secrete. The development of the mammary gland during the first part of pregnancy is under the hormonal influence of the ovaries, while during the second part the anterior pituitary hormone stimulates the gland to functional activity.

The experimental work which supports this knowledge was well reviewed by Nelson and Pfiffner.[5] These authors supplied further evidence by injecting lutein extract into immature female rats and observed marked development of the mammary gland. When a series of estrin injections was followed by a number of lutein treatments before the hypophyseal extract was employed in castrated or immature females, the glands enlarged and milk was secreted.

Riddle, Bates and Dykshorn [6] were able to extract this secretion-stimulating hormone from the anterior pituitaries of beef and named it prolactin. Allen, Gardner and Diddle [7] were able to induce lactation in ovariectomized monkeys by injecting theelin followed by galactin or prolactin.

First Lactation: The young mice are born on the 19th to 21st day of gestation. The first few days after parturition all the glands are in full function. The alveoli are comparable to a bunch of grapes — they are firm, round and white. Grossly they appear as uniformly distributed, small white granules which surround and obscure the ducts.

Microscopically sections show that the ducts near the nipple are dilated and contain milk. The lumens of the alveoli are large and contain droplets of secretion. The close contact of the epithelial cells with capillaries is evident and erythrocytes are seen in single layers within minute capillaries between the alveoli. Fibrocytes with flattened, deeply staining nuclei follow the course of these capillaries. The glandular parenchyma is in excess and adipose cells serve only to fill in the space left by it. As mentioned previously, the original lobes and lobules of the fat pad supply the framework of the lactating gland (Fig. 4).

The epithelial cells of the alveoli are not uniform but show all phases of activity. In some cells the nucleus is in the middle of the cell and the cytoplasm is homogeneous. In others the cytoplasm appears foamy and contains large protruding droplets. The nuclei have been pushed aside and flattened against the cell walls. Mitotic figures are rare although not entirely absent.

It was noticed after the 7th day of lactation in both the C57 black and the dilute brown animals that if the litter is small, some of the nipples are not suckled. The glands leading to these nipples differ from the nursed glands. The difference is noticeable grossly on the fixed skin. These glands are more yellowish in color as compared with the white, turgid suckled glands. The alveoli are shrunken and the enlarged ducts are plainly visible. Microscopically it is seen that these glands have undergone a certain degree of regression. The amount of adipose tissue is greatly increased, the ducts are distended with milk and the alveoli are decreased in size and number. The cells of the alveoli are irregular but many of them are functioning. Most often it is the first pair of glands

that is not used (16 out of 29 unsuckled nipples), but evidently it might be any of the glands (Figs. 5 and 6).

Cole [2] noted such regressions before weaning. In his material the 2nd and 3rd thoracic glands were most often neglected.

To ascertain that such regressive changes are really due to disuse, 2 of the nipples on the right side of a mouse lactating for 14 days were closed with celloidin. The animal was killed 5 days later and the glands of the right and left side were compared. The condition of the glands leading to the closed nipples proved that such regression is due to disuse. This emphasizes the importance of killing the animal and examining the condition of all the glands. Taking just part of a gland under anesthesia might lead to erroneous conclusions.

Microscopically little change in the structure of the glands during lactation in the 12 to 17 day period is seen. The development of the nursing young mice depends to a certain degree on the size of the litter. Consequently the mammary glands of a mouse nursing a large litter are still in full function at 3 weeks after parturition, while the glands of one nursing a small litter begin to show regressive changes at that time. The mother is usually separated from her young between the 3rd and 4th week after parturition, but the glands can be kept functioning for a prolonged period if nursing is continued by younger mice. In the glands of 3 of the dilute brown and 2 of the C57 black mice small areas of acute mastitis were observed showing polymorphonuclear leukocytic infiltration. The glands of 6 dilute brown and 6 black mice were included in this examination. They were between the ages of 2½ and 4½ months.

Regression after First Lactation: Regression is a reversed process of the changes that take place during pregnancy. Grossly there is a gradual decrease in the thickness of the gland. As the milk disappears from the alveoli the white granular appearance changes, the ducts become visible and the whole gland becomes yellowish in color. Microscopic sections show that 24 hours after suckling ceases, milk has accumulated in the ducts and alveoli, which become distended. A few epithelial cells are seen lying loose in the lumens. On the 2nd day of regression the distention of the ducts is further increased. The loosely lying epithelial cells undergo degeneration, the cytoplasm becomes swollen and the

pyknotic nucleus fragments. These cells are seen in increasing number as round, pink staining bodies with two or more deeply staining dots (Fig. 7). In some epithelial cells the swollen cytoplasm forms globules which are discharged into the lumen, but the nucleus with a small amount of cytoplasm remains intact. As the process of regression advances it is evident that the alveoli are gradually becoming smaller. On account of this they lose their close contact with the capillaries.

The lack of sufficient blood supply hastens the process of regression. The space between the shrinking alveoli is being filled by adipose cells. Some of these cells seem to develop from the fibroblasts which are in close proximity to the capillaries. In the lactating gland these cells have an elongated, deeply staining nucleus and very little cytoplasm. At the beginning of regression they begin to change. The nucleus becomes rounded and the amount of cytoplasm increases. Gradually fat accumulates in the cytoplasm and the nucleus is pushed to the periphery. During this change the size of the cells increases immensely in all dimensions. The increase in the amount of adipose cells is first noticeable interlobularly and later intralobularly.

Four days after lactation stops the alveoli are much smaller and are very irregular in outline. Some of the alveoli are collapsed and form irregular clumps of cells. In others the cytoplasm is still swollen. There are a few degenerating cells which have pyknotic and fragmenting nuclei. The lumens of several alveoli are still filled with secretion but the globules have disappeared and the secretion is homogeneous. The main ducts proximal to the nipples are still somewhat enlarged. They contain a few degenerated cells and secretion. The amount of adipose tissue is greatly increased.

Six days after the discontinuation of nursing most of the alveoli are collapsed and form irregular groups of cells. The nucleus is small and compact — the cytoplasm is no longer swollen. Several of the cells have pyknotic, a few of them fragmenting, nuclei. The clumps of epithelial cells are surrounded by fibrous connective tissue. After the first lactation period is over the amount of the fibrous connective tissue surrounding the parenchyma is more compact than before. The amount of adipose tissue increases proportionately with the regression of the parenchyma. Most of the adipose cells have attained full size by now, but some of them are

still in the process of expansion. The epithelial cells of the terminal ducts undergo the same changes during regression as the alveoli. The lumens of the ducts decrease greatly in size, but the epithelial cells lining it retain their orderly arrangement.

On the 9th to 11th days of regression the few alveoli which are still present consist of small, compact, circularly arranged cells containing deeply staining nuclei and very little cytoplasm. They are closely grouped around the ducts and are surrounded by fibroblasts and a large amount of adipose cells. The main ducts proximal to the nipple are still slightly dilated and contain secretion and a few dead cells.

About 11 to 12 days after nursing stops regression is usually complete. The ducts proximal to the nipple have larger lumens than previous to the first lactation. Distal to the nipple the lumens become smaller, finally consisting only of rows of cells in the terminal branches. There are no alveoli. The gland can be now considered a "resting" gland (Fig. 8).

The process of regression is not very uniform. Considerable variation exists between the glands of the same animal, depending on whether the nipple was suckled or not up to the time of weaning.

The examination of the glands of 5 dilute brown and 4 black mice is included in this group. In the glands of 1 dilute brown and 1 C57 black animal small areas of acute mastitis were found. All the mice were between the ages of 3 to 5½ months. No significant structural differences were found between the dilute brown and the C57 black strains of mice.

It was during the period of second pregnancy that some interesting abnormalities were found in the dilute brown strain. These will be described in more detail.

Second Pregnancy: With the beginning of the second pregnancy there is again a period of rapid development of the glandular parenchyma. The rate of growth increases, as during the first pregnancy.

At 7 days pregnancy the changes were similar in both strains to the development taking place during the corresponding period of the first pregnancy.

At 15 days pregnancy in dilute brown No. 4540 abnormal areas were observed in the 3rd gland. The areas were small and were

not noticeable grossly or under the dissecting microscope. The general development of all the glands was quite uniform. Mitotic figures were rare; the epithelial cells of the alveoli were undergoing hypertrophy and were beginning to secrete. In the abnormal areas the epithelial cells did not show hypertrophy and functional activity but remained small and compact and showed numerous mitotic figures. Some alveoli and the ducts leading to them had no lumens but were composed of solid cords and groups of cells. While most of the cells appeared to be limited by a basement membrane, some of them were beginning to invade the surrounding tissue, become disorganized and show definite signs of carcinoma. At such areas the cells were slightly larger and stained lighter. The nucleus was less compact and the amount of cytoplasm was increased. The cellular density of the surrounding stroma was increased (Figs. 9 and 10).

Dilute brown mouse No. 5430 had been pregnant the second time for 19 days. Macroscopic abnormalities could not be observed. Microscopically uniformly developed glands which were composed of secreting alveoli were seen. An abnormal area was present on the 1st cervical gland and involved a whole small lobule. Secreting cells were absent in this area, but mitotic figures were numerous among the epithelial cells as well as among the fibrous connective tissue cells of the stroma. The arrangement of the epithelial cells was not atypical and the area did not show a carcinomatous structure although physiologically the cells seemed to be out of normal control, persisting in cell multiplication, while in the cells of the surrounding area secretion had started (Figs. 11 and 12).

The changes in the glands of 1 other dilute brown and 5 C57 black animals examined during the second pregnancy were comparable with those described at the first pregnancy and showed no abnormalities. All the mice in this group were between the ages of 4 and 5 months.

Second Lactation: Three of the dilute brown animals examined while suckling their second litter had normal lactating glands. One dilute brown which was lactating 7 days (No. 5636) showed an area of chronic mastitis on the distal part of the 4th gland. The ducts were densely infiltrated with polymorphonuclear leukocytes. The surrounding area showed lymphocytic infiltration.

Some of the alveoli were obliterated and the stroma showed fibrosis. Three of the C57 black animals had normal lactating glands.

In 1 of the black animals which was lactating 6 days (No. 10526) the 1st nipple was filled with cellular débris which evidently caused obstruction. The primary duct leading to this nipple was greatly distended while the rest of the gland structure showed regression due to disuse.

It was observed in both strains during this period, as in the period of first lactation, that the glands that were not suckled were undergoing regression. Again the 1st nipple and gland structure leading to it were most often subject to disuse.

All 4 dilute brown and 4 black mice examined while lactating their second litter were between the ages of 4 and 6 months.

Regression and Resting after Second Lactation: Dilute brown No. 5432 was killed 3 days after her young were weaned. The glands showed much irregularity. Evidently several of the glands stopped functioning considerably sooner. The 2nd gland was the one least regressed. This gland was similar in every respect to that showing the early regressive changes described before. A small abnormal area was observed on the 5th gland. It consisted of a very small lobule in which the alveoli did not show any regression but contained numerous mitotic figures. Although the epithelial cells did not show secretory activity the lumens contained dense, deeply staining secretion. The area was not malignant but was definitely different from the areas which can be considered "normal" at this stage. The glands of 3 other dilute brown animals were examined in a resting state. One of them (No. 3214) showed an area of acute mastitis in the 5th gland. All of them showed a few small groups of alveoli which persisted through the regressing period.

The glands of 3 C57 black animals were examined and showed uniform normal regression. All the animals examined during this period were between the ages of 6 and 8 months.

Third Pregnancy: Dilute brown No. 6481 which was pregnant for 7 days showed the usual increased mitotic activity and rapid development of alveoli. There were a few small lobules which in contrast with this consisted of fully developed alveoli, the epithelial cells of which showed functional activity. One of these

lobules which was found on the 1st gland contained a few alveoli in which the epithelial cells had undergone metaplasia, changed into stratified squamous cells and filled the lumens with cornified cell débris (Fig. 13). The stroma surrounding this area showed fibrosis and some lymphocytic infiltration. It is probable that these functioning areas persisted since the previous lactation. Two small areas of acute mastitis with dense polymorphonuclear leukocytic infiltration were also observed on this gland. Dilute brown No. 10807 which was pregnant for 9 days had a large abscess surrounded by a fibrous capsule on the 3rd thoracic gland. A small area showing metaplasia and cornification of epithelial cells was found on the 5th gland. In the fibrous stroma which enclosed this area polymorphonuclear leukocytes and lymphocytes were observed. The rest of the glands showed the usual rapid development, which is characteristic at this stage of pregnancy. Two more dilute brown females pregnant for 15 and 19 days respectively showed normal gland structures.

The 3 C57 black animals examined during their third pregnancy did not show any structural abnormality. All these mice were between the age of 7 and 9½ months.

Third Lactation: Dilute brown No. 92675 had been nursing for 7 days. As there were only 2 young in the litter, all the glands were not suckled and showed irregular regression. In the 1st, 3rd and the 5th nipple the primary duct was greatly dilated and was full of milk. The amount of adipose tissue was increased. The alveoli showed much variation in size and activity but most of them were in the process of regression. Several small areas showed chronic mastitis with fibrosis and lymphocytic infiltration. The 2nd and the 5th glands were lactating normally. Another (dilute brown No. 2816) was also suckling 2 young mice only and showed regression, due to disuse in several glands. Similar irregularities and abnormalities that were described in the glands of the previous mouse were found in this gland too. In a 3rd (dilute brown No. 97374) the 1st nipple was infested with mites. They evidently penetrated to a short distance into the lumen of the nipple and were sectioned lying within the lumen. The epithelial lining showed signs of irritation — increased amount of keratinization and lymphocytic infiltration.

One of the C57 black animals examined at this stage had fully

functioning normal glands. Three others showed 1 or more glands in the process of regression. In these regressing glands the ducts proximal to the nipple were distended, the amount of adipose tissue was increased and the alveolar epithelium showed irregularities. In 1 mouse (No. 14403) a small area of acute mastitis was noted. All these animals were between the ages of 8 and 11½ months.

Resting after Third Lactation: All the glands of the dilute brown and black animals examined in this group completed their regression and were in a resting stage. In all of them the adipose tissue was greatly increased and the epithelial elements were at their minimum. Three dilute brown animals showed a few scattered groups of alveoli in several of their glands where the secretory activity persisted. Such areas were surrounded by normal adipose cells.

Dilute brown No. 4353 had a tumor on the 3rd gland which although small was large enough to be palpable. Microscopic examination showed an adenocarcinoma with hemorrhagic cysts and some necrosis. The adenoid structure consisted of small alveoli and had some resemblance to the glandular structure of a gland at the first half of pregnancy. The glands of this animal also contained several small areas where groups of alveoli persisted without completing their regression.

Two C57 black mice showed normal resting gland structure. All mice in this group were between the ages of 7 and 8 months.

Fourth Pregnancy: Dilute brown No. 4127 had been pregnant for 7 days when killed. A microscopic abnormal area was found on the 2nd gland. It consisted of an increased amount of epithelial cells changing and enlarging the normal outline of a few small ducts and alveoli. The area was surrounded by fibrocytes. Mitotic figures were numerous (Fig. 14).

Dilute brown No. 8095 had been pregnant for 10 days. A small palpable tumor was noticed on the 1st gland. Microscopic sections showed a rapidly growing adenocarcinoma with many mitotic figures.

Adjacent to this nodule there were some alveoli that contained secretion. The presence of a few small lobules with persistent secretory activity was noted on the 3rd and 4th glands.

Dilute brown No. 4165 was pregnant for 19 days and all the

glands consisted of well developed, functioning cells. On the 4th gland a microscopic area was observed where the epithelial cells were not functioning but rapidly dividing. In some places the lumen was being obliterated by an overgrowth of epithelial cells. While in most places the basement membrane was intact, in some places it had been broken through and the epithelial cells were seen in the process of invading the surrounding tissues and showed definite signs of carcinoma. The stroma was fibrous (Figs. 15 and 16).

One black animal examined at the corresponding stage showed acute mastitis in 1 gland. Two others proved to be normal. The animals included in this group were between the ages of 8 and 13 months.

Fourth Lactation: Dilute brown No. 3951 had no palpable tumor, but suspicious areas were noticed under the dissecting microscope on the left 1st and right 3rd glands. Sections showed that all the glands were lactating. The suspicious area on the left 1st gland was a small fibroadenoma. The stroma was very dense and at one place near the periphery showed lymphocytic infiltration. The small nodule on the right 3rd gland was an adenocarcinoma with fibrous stroma. The tumor was not encapsulated and was in the process of expansion by peripheral growth (Fig. 17).

All the glands of 2 other dilute brown and 2 C57 black animals examined at this stage were normal lactating glands. These mice were between the ages of 8 and 9½ months.

Resting after Fourth Lactation: Dilute brown No. 2585 showed acute mastitis with diffuse polymorphonuclear leukocytic infiltration on the 5th gland. In all other glands the regression was normal and uniform.

On the 4th gland of dilute brown No. 2796 a small area was considered suspicious under the dissecting microscope. After sectioning it was found to be composed of atypically arranged ducts and alveoli of greatly varied size. Many of these were composed of solid cords of cells and several of them had broken through the normal boundaries and were definitely malignant. Mitotic figures were numerous. The stroma showed slight fibrosis and lymphocytic infiltration. The area was not encapsulated (Fig. 18). On the 3rd gland a small area showed signs of chronic inflammation. In some of the alveoli in this area the epithelial cells changed into

cornified stratified squamous cells, due to metaplasia. All of the glands contained some small persistently secreting groups of alveoli.

The glands of 2 black mice were examined at this stage. All showed normal structures. These animals were between the ages of 9 and 12 months.

Fifth Pregnancy: Dilute brown No. 7110 was pregnant for 8 days. Microscopically the sections showed several small areas where aveoli containing quite dense secretion persisted, evidently from the previous lactation period. The main ducts in the 1st and 5th glands were distended by retained secretion. In the 2nd gland a very small, definitely malignant area was observed. This area showed some rapidly and densely growing epithelial cells without alveolar arrangement, developing around a small papilliferous cystic adenocarcinoma. The stroma was fibrous but the area was not encapsulated. None of the elements in the malignant area contained retained secretion. A somewhat larger malignant area was present in the 5th gland. This area was noticed under the dissecting microscope but was not large enough to be palpable. Here the epithelial elements were arranged in adenoid form. Some alveoli contained retained secretion; others contained no secretion and were growing very rapidly. In a few of them near the periphery calcium deposit was found. Several small cysts contained necrotic material. The stroma was quite fibrous; the tumor was not encapsulated.

Dilute brown No. 7856 was pregnant for 9 days. In the 5th gland a small group of ducts and developing alveoli were growing atypically. The epithelial cells were rapidly multiplying and were lining the lumens with several rows of cells. The basement membrane was still intact. The surrounding stroma showed increased fibrosis. The rest of the glands did not show any abnormalities.

Dilute brown No. 8061 was pregnant for 15 days. The development of all the glands was quite uniform. A few ducts contained some retained secretion. In the 4th gland a small abnormal nodule was noticed microscopically. It was a carcinoma simplex and consisted of a disorganized growth of large atypical epithelial cells. The nodule was surrounded by a fibrous capsule which showed some lymphocytic infiltration (Fig. 19).

Dilute brown No. 7869 was pregnant for 19 days. All the glands

TABLE I

Dilute Brown

Number of animal	Age in months	Abnormalities
	First Pregnancy	
2664	2	
2819	−3	
2483	−4	
2808	3	
2112	3	
2662	−3	
2502	2+	
	First Lactation	
78335	3	
2878	3+	Small areas of acute mastitis
78261	4	
2887	3+	
2883	3+	Small areas of acute mastitis
2916	3	Small areas of acute mastitis
	Regression after First Lactation	
2815	4	Small areas of acute mastitis
4187	4	
4108	4	
5535	−5	
97371	5½	
	Second Pregnancy	
4540	5	Adenocarcinoma
5430	5	Persistent growing area
1688	5	
	Second Lactation	
96624	−6	
5636	6	Chronic mastitis
3310	4	
3320	4	
	Regression and Resting after Second Lactation	
5432	8	Persistent growing area
3425	6	
3214	−7	Acute mastitis
3003	7	

Number of animal	Age in months	Abnormalities
	Third Pregnancy	
6481	7	Metaplasia, chronic and acute mastitis
3041	7	
3283	7	
10807	9½	Abscess, chronic mastitis and metaplasia
	Third Lactation	
92675	−11	Chronic mastitis
2816	11½	
97374	6	Infestation with mites
	Resting after Third Lactation	
2826	8	Small areas of persistent secretory activity
2818	8	Same as above
10806	8	Same as above
4353	8	Adenocarcinoma
	Fourth Pregnancy	
4127	8	Thickened wall of ducts
8095	−13	Adenocarcinoma, persistent secretory activity
4165	−9	Adenocarcinoma
	Fourth Lactation	
3951	9	Fibroadenoma Adenocarcinoma
3882	9	
4006	8	
	Resting after Fourth Lactation	
2585	9	Acute mastitis
2796	9	Adenocarcinoma, chronic mastitis and metaplasia
	Fifth Pregnancy	
7110	13	Carcinoma simplex, adenocarcinoma
7856	12	Thickened wall of ducts and alveoli
8061	12	Carcinoma simplex, persistent secretory activity
7869	13	Fibroadenoma

were composed mostly of secreting epithelial cells. A small fibroadenoma was found on the 5th gland. The epithelial cells filled up the lumens and enlarged the glandular structure. The basement membrane was not broken through. Mitotic figures were numerous. The stroma showed increased fibrosis. The tumor was not encapsulated (Fig. 20).

TABLE II

C57 Black

Number of animal	Age in months	Abnormalities
	First Pregnancy	
14135	−2	
14151	2+	
13921	2+	
14277	2+	
14242	2+	
14312	2+	
	First Lactation	
14328	2+	
14327	2+	Acute mastitis
13594	4+	
13695	4+	
13920	3+	Acute mastitis
11907	4	
	Regression after First Lactation	
15602	4	
15334	4	
15628	4	Acute mastitis
21484	3	
	Second Pregnancy	
146	5	
147	5	
280	5+	
281	5	
282	4+	
	Second Lactation	
15664	4	
15983	4	
15888	−5	
10526	−5	Obstruction of 1 nipple

Number of animal	Age in months	Abnormalities
	Regression and Resting after Second Lactation	
14040	8	
14248	7	
14313	7	
	Third Pregnancy	
15643	8	
16218	8	
283	8	
	Third Lactation	
14403	8	Acute mastitis
16051	8	
16340	7	
1192	10	
	Resting after Third Lactation	
14136	8	
14153	7+	
	Fourth Pregnancy	
18609	13	Acute mastitis
14702	10	
19318	11	
	Fourth Lactation	
14440	9	
2768	9½	
	Resting after Fourth Lactation	
15357	12	
14202	10	
	Fifth Pregnancy	
19312	12	
19313	−12	
19838	11	

Comparing the glands of these dilute browns with the glands of 3 blacks which were included in this group, the unevenness in the development of the dilute brown mice is becoming more and more evident. All these mice were between the ages of 11 and 13 months.

Murray [8] made a detailed analysis of the breeding behavior of the dilute brown strain. According to his data the frequency distribution curve representing the age of mice at the appearance of tumor reaches its mode at 10½ months. As the animals described in the last group had already passed through this age the investigation has not been carried further.

The age of the animals and the abnormalities observed in them from the period of the first to the fifth pregnancy are presented in Tables I and II.

Discussion

From the data presented above it is evident that comparing the mammary glands of the dilute brown high tumor strain with the C57 black low tumor strain, structural differences can be detected before the appearance of palpable tumors. These differences manifest themselves in two main ways:

1. During the latter part of pregnancy a uniform change takes place in the epithelial cells of the glands of the black animals — cell multiplication ceases and secretion starts.

In the dilute brown mice this change is not always uniform and small groups of epithelial cells sometimes persist in cell multiplication.

2. During the period of regression the epithelial cells of the glands of the black animals gradually cease to secrete and the alveoli regress completely.

In the dilute brown mice these changes are not always uniform — small groups of alveoli often persist and sometimes some of the epithelial cells keep on secreting.

As mentioned previously, enough experimental data have accumulated to prove that although breast development is brought about during pregnancy by estrin and luteal hormones, the secretion of milk can be effected only through the agency of an anterior pituitary hormone — prolactin. It seems important that a proper balance should be maintained between the hormones that stimulate

cell division and function. This balance might be upset in several ways. (*a*) One or the other hormone might be produced in excess quantity. In this case the effect of the resulting disturbance would probably be more widespread. (*b*) Some groups of cells might be able to store up or unable to eliminate certain hormones. This might render them incapable of utilizing the effect of other hormones.

In a recent paper Geschickter and Lewis [9] present an interesting study of pregnancy and lactation changes in fibroadenoma of the breast in humans and state: "The failure of the fibroadenomatous tissue to respond to the same extent and in the same manner as normal breast tissue to all phases of pregnancy and lactation suggests that the tumor is more sensitive to certain hormones than to others." In the present study this "failure to respond" was found to be present in certain groups of cells even before the cells were atypical or produced excessive growth.

The failure of small groups of epithelial cells to respond to the stimulation of prolactin and persist in cell multiplication during the latter part of pregnancy seems to lead to carcinoma in the dilute brown animals.

Structurally these persistently growing areas are composed at an early stage of small epithelial cells with a relatively large nucleus and a small amount of cytoplasm surrounding a small lumen. Mitotic figures are numerous. The surrounding stroma shows increased fibrosis. Later the epithelial cells fill up the lumen, enlarge the whole outline of the duct and finally break through the basement membrane. Although the fibroblasts which surround these areas at the early stages also show proliferation, they soon lag behind in development and the epithelial cells often penetrate into the surrounding adipose tissue. Cyst formation if present at all in this type of tumor is usually due to necrosis or rupture of blood vessels.

The failure to complete regression and the persistence of secretory activity of small groups of epithelial cells do not seem to be so dangerous from the point of view of malignant changes. In such areas mitotic figures are infrequent. If malignant changes of these areas do take place they probably develop slowly and later in the life of the animal.

Metaplasia was noted in several of the glands of the dilute

brown mice. Cornified cells filled the lumens and the surrounding area showed fibrosis and signs of chronic mastitis.

Small areas of acute mastitis were observed most often during lactation and were found in the glands of the black animals as well as in the dilute brown animals.

Summary and Conclusions

A comparative morphological study of the mammary glands of the Little-Murray dilute brown high tumor strain and the C57 black low tumor strain of mice showed that the glands of the high tumor strain do not respond so uniformly to the endocrinal influences that regulate the progressive, functioning and regressive changes of the gland as do those of the low tumor strain.

In the high tumor strain groups of cells may persist in cell division, while all the others are already functioning; or fail to regress, sometimes keeping on functioning while all the others have undergone regression. The persistent mitotic activity of groups of cells leads to early malignant changes.

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DESCRIPTION OF PLATES

PLATE 135

FIG. 1. End bulbs on the mammary gland of an 8 weeks old dilute brown mouse. × 65.

FIG. 2. Rapidly developing mammary gland on the 11th day of pregnancy. × 200.

FIG. 3. Mammary gland showing secretory activity on the 20th day of pregnancy. Note the decrease of adipose cells and the increase of the glandular elements. × 200.

FIG. 4. Mammary gland of a mouse lactating for 7 days. Note the almost complete absence of adipose cells. × 100.

FIGS. 5 and 6. Glands of the same C57 black mouse that was lactating for 20 days. Figure 5 is the 4th gland and shows secretory activity. Figure 6 is the 3rd gland and shows regression of the glandular elements and an increase of adipose tissue. × 50.

FIG. 7. Mammary gland 24 hours after lactation had stopped. Note the round bodies in the ducts and alveoli. × 100.

FIG. 8. Mammary gland at resting stage. Adipose cells greatly increased — the glandular elements reduced. × 100.

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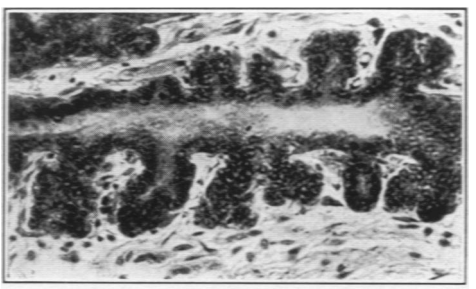

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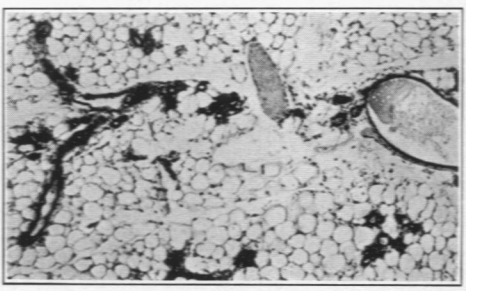

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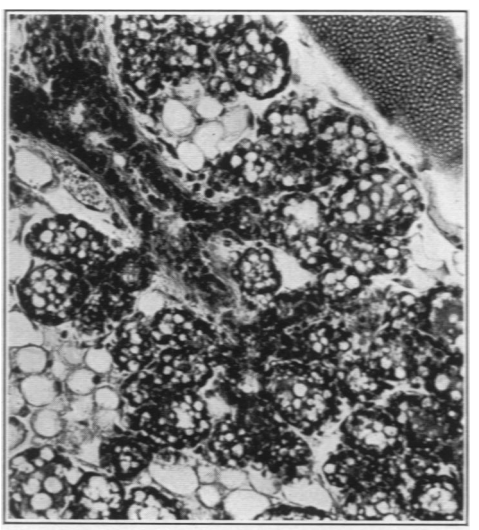

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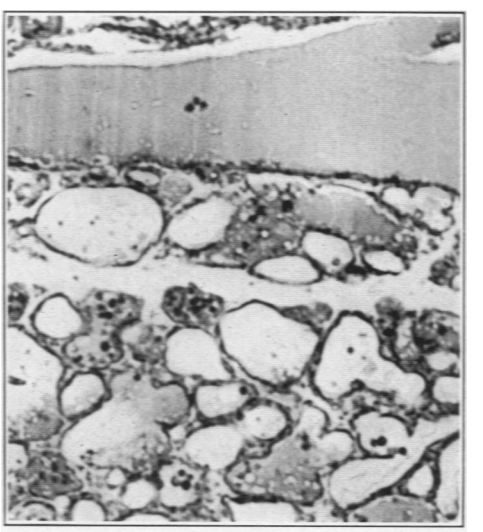

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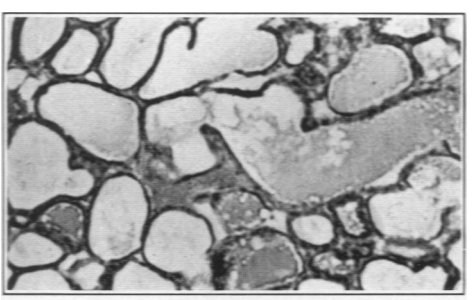

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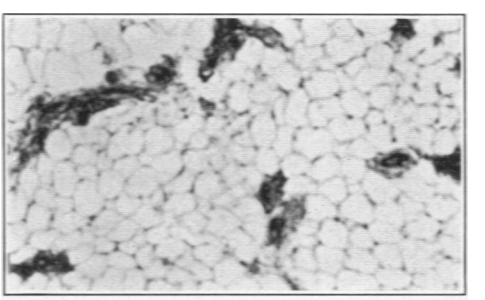

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Mammary Gland in High and Low Tumor Strain

PLATE 136

FIG. 9. Mammary gland of dilute brown No. 4540. This animal was pregnant for 15 days and secretory activity has started in the cells of the normal area. The abnormal areas are deeply staining and do not show secretory activity. × 25.

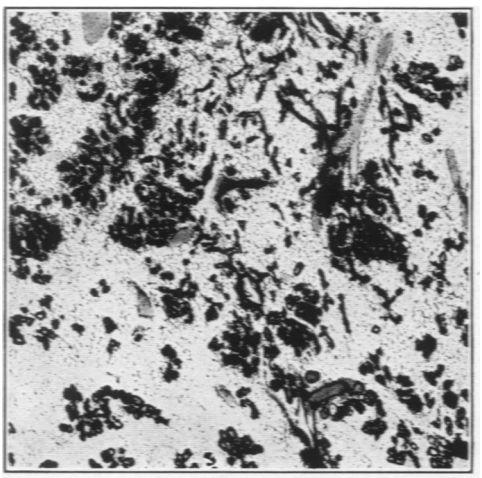
FIG. 10. Part of Figure 9 under higher magnification. In the lower left corner a normal lobule is seen. The upper right corner shows malignant changes in the abnormal area. × 250.

FIG. 11. Mammary gland of dilute brown No. 5430. The animal was pregnant for 19 days and secretory activity was well established. In the abnormal area the cells are not functioning but are still actively dividing. × 25.

FIG. 12. Part of Figure 11 under higher magnification showing normal part at the lower left and abnormal part above it. × 75.

FIG. 13. The gland of dilute brown No. 6481 at 7th day of pregnancy. The abnormal area shows a few alveoli with persistent, functional activity and metaplasia of the glandular epithelium to cornified, stratified squamous epithelium. × 100.

FIG. 14. Dilute brown No. 4127 pregnant for 7 days showing the thickened wall of a few ducts and alveoli. × 75.



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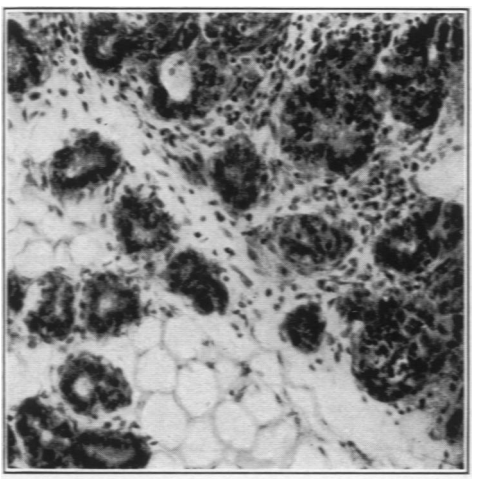

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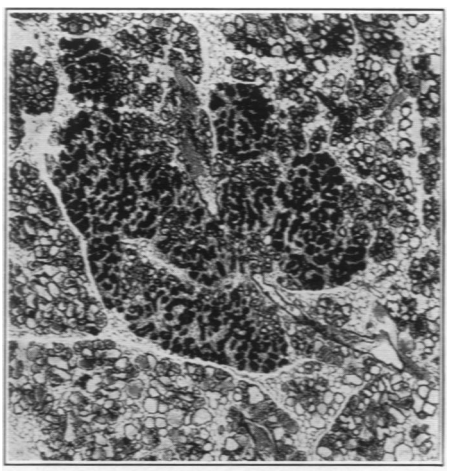

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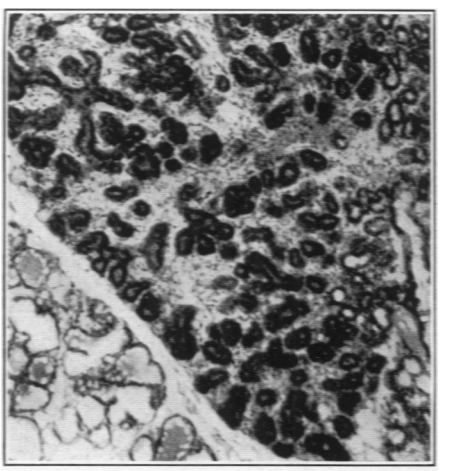

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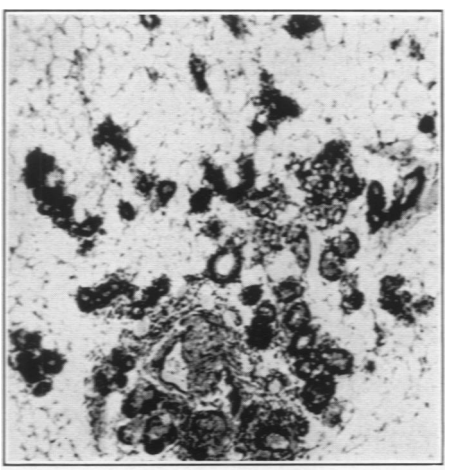

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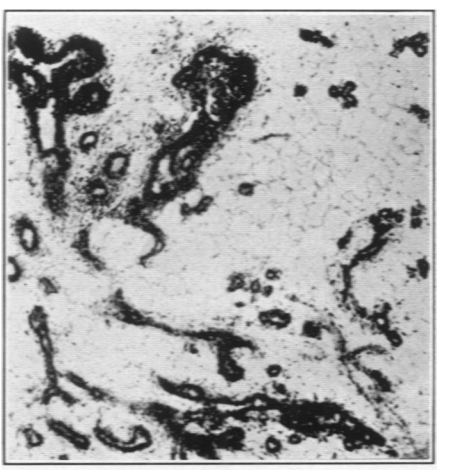

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Mammary Gland in High and Low Tumor Strain

PLATE 137

FIG. 15. Dilute brown No. 4165 pregnant for 19 days showing an abnormal growing area surrounded by a normal functional area. × 25.

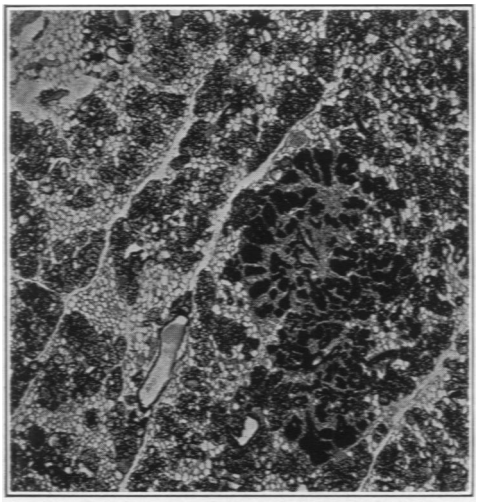
FIG. 16. Part of Figure 15 under higher magnification. The normal area is on the left; the abnormal on the right side of the picture.

FIG. 17. The gland of dilute brown No. 3951 lactating 1 day shows an adenocarcinoma with fibrous stroma. × 75.

FIG. 18. The gland of dilute brown No. 2796 at resting stage, showing a small adenocarcinoma. × 25.

FIG. 19. The gland of dilute brown No. 8061 pregnant for 15 days and showing a small carcinoma simplex which is encapsulated. × 100.

FIG. 20. The gland of dilute brown No. 7869 pregnant for 19 days and showing a fibroadenoma. × 25.



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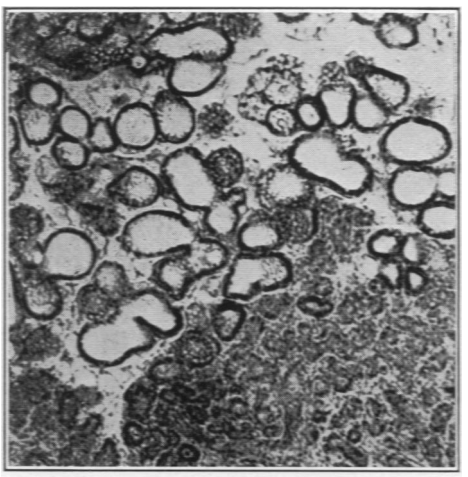

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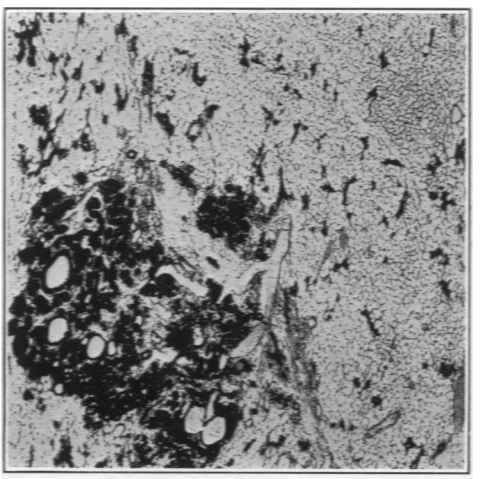

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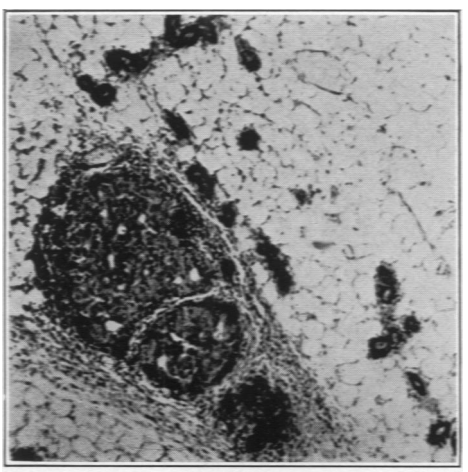

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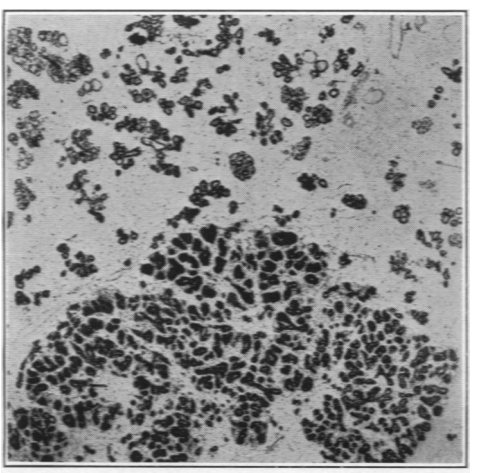

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Mammary Gland in High and Low Tumor Strain