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CHANGES IN THE NATURE OF THE STROMA IN VAGINA, CERVIX AND UTERUS OF THE MOUSE PRODUCED BY LONG-CONTINUED INJECTIONS OF ESTROGEN AND BY ADVANCING AGE [1]

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In the course of our studies of the effects of estrogen on proliferative changes in the mammary gland, vagina, cervix, and uterus of the mouse, we noted certain marked and unexpected changes in the character of the stroma of the three last named organs. These changes are distinct in certain respects from the fibrosis mentioned by Lacassagne (1) as following long-continued injections of estrone. They are of twofold interest. In the first place, we know that a constant interaction takes place between stroma and parenchyma, and that the state of the stroma may affect directly or indirectly the state of the epithelial structures and also the growth processes which may here take place. Conversely, it can be shown that growth processes in the epithelial structures may affect those in the stroma. We may also recall the fact that in one instance we observed the development of a sarcoma in the stroma of the vagina and cervix in a mouse injected with estrogen over a long period. Secondly, in the analysis of old age changes in animals, alterations in the character of the stroma are found to be very important. On the nature of the stroma which surrounds blood and lymph vessels depend largely the function and structure of the epithelial elements. From these points of view we have analyzed the changes observed in the stroma of approximately 500 mice which we had previously studied for other purposes. In a paper on growth processes induced in the uterus of mice by injections of estrogen we have classified these mice in regard to age and to the amount of estrogen and other hormones with which they were injected (2).

[1] These investigations were carried out with the aid of grants from the International Cancer Research Foundation and from the Committee for Research on Endocrinology of the National Research Council. We are greatly indebted to Dr. Erwin Schwenk of the Schering Corporation for supplying us with Progynon B (benzoic acid ester of dihydrofolliculin), the estrogenic substance used in these experiments.

I. The Stroma of Vagina, Cervix and Uterus in Normal Mice

From Birth to the Age of Six Months: There is in the vagina and cervix of normal mice a gradual slight increase in the relative amount of connective-tissue fibrillae from the age of three weeks to the age of four months. At the age of one month the stroma of the vagina is mainly cellular; between the cells there is some loose collagen. At three months a rather thin layer of loose, homogeneous material enclosing connective-tissue cells was found beneath the surface epithelium. In a mouse four months old there was an increased amount of homogeneous collagenous substance beneath the epithelium and this extended into the outer parts of the stroma in the direction of the muscle tissues.

We shall in a general way designate the homogeneous collagenous material which is deposited, or which may otherwise develop between the connective-tissue cells, as hyaline, although in a strict sense this term is applicable only to homogeneous fibrous tissue of a very dense consistency and glassy appearance. All transitions exist, however, from this material to a softer gelatinous substance in the stroma and also in the circumference of blood vessels—especially arteries—and of gland ducts or fundi, and beneath the surface epithelium. A small amount of hyaline material may also develop at this age around the blood vessels. On the whole the collagenous part of the stroma at this period is moderate in amount and not dense, and the latter, in the portio and around the folds, particularly their inner walls, is of a rather cellular character.

Passing from the vagina by way of the folds to the cervix and then to the uterus we find a gradual and continuous increase in richness in connective-tissue cells and a decrease in the amount of fibrillar-fibrous elements. In the uterus the stroma is rich in cells, which may be separated by thin fibrils of collagen. The nearer the cells are to the surface epithelium, the fewer are the fibrils separating them; in the direction of the muscle tissue the collagenous material increases and in the region adjoining the inner muscle layer it reaches a maximum. Hyaline substance is usually not found at this age period, but in mice five or six months old a slight amount of it may be seen around some of the vessels which are situated between the two muscle layers.

Ovariectomy seems to increase the hyaline character of the stroma in the vagina, especially in the lower portion near the entrance, but there may be an increase in hyaline substance also about the folds, in the zone between folds and cervix, and affecting the muscle tissue in this region. Furthermore, there may be an increase in the amount of intercellular collagen in the part of the cervix which adjoins the vagina. In the uterus of ovariectomized mice, on the contrary, there is a decrease in the intercellular collagen and the stroma here consists largely of small connective-tissue cells, packed together. Such a condition is especially noticeable in the zone lying directly beneath the surface epithelium, while in the deeper parts of the uterine wall near the muscle layers connective-tissue fibrils may again become more prominent. This mode of response of the uterine wall to ovariectomy, in which the stroma participates, is presumably due to the atrophy caused by loss of ovarian hormone action. In accordance with this interpretation we find that the uterine wall as a whole, and in particular the muscle layers, are thin. The removal of the

normal stimulation exerted in the vagina, cervix, and uterus by hormones, especially estrogen, thus seems to lead to different results: in the vagina and cervix a fibrous-hyaline condition predominates and in the uterus the characteristic mode of reaction is atrophy.

From Six to Twelve Months: Between the ages of six and twelve months the condition of the stroma in the vagina and cervix has been found, on the whole, to be similar to that in the earlier period. Allowance must be made, however, for certain individual variations; in some of the mice eleven to twelve months old there may be a slight increase in the amount of collagenous substance, which in some cases extends into the muscle layer. As usual, in the cervix and in the portio the connective tissue is more cellular than in the vagina; but in these places also there may be some increase in the amount of collagenous intercellular substance.

The stroma of the uterus, likewise, may not differ very much from the stroma seen at the earlier period. Again, in some mice ten to twelve months old we may observe a slight increase of hyaline substance around some of the glands, as well as in the stroma situated near the inner muscle layer and around the arteries running between the muscle layers. In this respect there exist variations in different mice, but on the whole no very marked alterations have taken place as compared with the preceding period. This might be expected in view of the cyclic changes occurring in vagina and uterus during this period, which affect the epithelium as well as the connective tissue, and are responsible for considerable individual differences in accordance with the stage of the sexual cycle at the time of examination of the animal. We do not wish to exclude the possibility that added to these differences there may be a steady slight increase in fibrillar-fibrous material, but at the present stage of our investigations we are not yet able to state more definite conclusions.

Furthermore, *in ovariectomized mice* the stroma of the vagina appears, on the average, more dense, while in the uterus the tissue between surface epithelium and muscle is packed with small nucleated cells, although in some cases there is an increase in fibrillar or slightly hyaline stroma under these circumstances.

From Thirteen to Eighteen Months: In mice thirteen to eighteen months old a slight increase is found in the average amount of fibrillar or fibrous-hyaline connective tissue in the vagina as well as in the uterus, and as a rule hyalinization becomes more marked during the latter part of this period. Considerable variations exist, however, in individual animals. In the vagina the character of the stroma may in some cases still be fibrillar-cellular, but usually a greater amount of fibrous-hyaline tissue is deposited between the cells and this substance often extends into the muscle tissue; in addition, densely hyaline material is frequently observed around the folds, and hyaline changes are seen occasionally in the tissue situated between the inner wall of the fold and the cervix. In the cervix the connective tissue is usually richer in cells, which are separated by fine fibrils, but here also there is a gradual increase in the average amount of hyaline material, which may penetrate into the underlying muscle tissue.

In the uterus the character of the stroma is for the most part fibrillar-cellular, but in this organ also an increase in the intercellular fibrillar sub-

stance occurs and hyaline material may be deposited between the cells. In some cases the hyaline may be of dense consistency, may penetrate into the inner muscle layer, and in addition surround some arteries. In a few instances hyaline rings were found around a few uterine glands. While there is, then, on the whole, an increase in fibrillar and also of homogeneous hyaline connective tissue at this age period, it is usually relatively slight, though variations occur in this respect in different individuals.

After Eighteen Months: Above the age of eighteen months, and especially beyond twenty months, the number of mice in which the stroma is markedly fibrous or hyaline in vagina and cervix is distinctly increased. Here again it is especially the connective tissue about the folds which may be densely hyaline, while the connective tissue of the portio also may now show a fibrous-hyaline character. Furthermore, the connective tissue as well as the muscle tissue of the cervix, and especially the area between folds and cervix, may undergo hyalinization, and around the arteries in the deeper tissues of the cervix hyaline may be deposited. But here, too, some variations are found in different animals. In a mouse thirty-six months old, for example, the hyaline in the vagina was only moderate in amount and there was none in the cervix. Perhaps atrophic processes which occur in old age may in some instances counteract the tendency to the deposit of dense hyaline tissue in these organs.

In the uterus, also, variations are observed in the character of the stroma in individual mice, but as a rule there is an increase in fibrillar and in hyaline connective tissue surrounding the cells. As usual, the fibrillar or fibrous-hyaline tissue is observed in greater quantity in the deeper tissue, adjoining and even infiltrating the muscle, than in the zones beneath the surface epithelium. There may be an increase in hyaline material, also, around the arteries between the layers of muscle tissue. While in some animals rather extensive hyalinization may take place in the deeper areas of the connective tissue, more commonly the amount of hyaline substance remains moderate and the number of fibrocytes which are imbedded in it is considerable.

With the stroma of normal mice, we shall now compare the changes produced by long-continued injections of various quantities of estrogen.

II. The Stroma in Mice Injected with 1–30 Rat Units of Estrogen over Long Periods

There is, on the average, an increase in the amount of fibrous-hyaline connective tissue in the vagina, cervix, and uterus of the mouse following injection of estrogen in such relatively moderate quantities as 1 to 30 rat units. The degree of fibrous-hyaline change which occurs under these conditions is determined by several factors. (1) The effect increases, on the whole, with increasing length of the period of injections and therefore also with increasing age of the injected mice. (2) The effect is the greater, the larger the doses of estrogen used and the more frequently they are given. (3) There exist in addition individual and perhaps also strain variations. The same dose of estrogen given over the same length of time does not produce in all mice the same effects. In some animals the stroma may be merely fibrillar-cellular, while in others much fibrous-hyaline material is deposited in the area between

surface epithelium and musculature. Furthermore, it seems that in mice belonging to the D strain the arteries are perhaps less prone to undergo hyaline changes than in animals belonging to the A, the C57, and some other strains. Even in D mice of eighteen months or older a marked hyalinization in uterus and cervix was lacking. Whether we have actually to deal in these cases with a strain peculiarity remains, however, to be determined.

As to the character of the changes in the injected animals, they are usually of the same nature as those which were noted in the control mice, but the average degree of hyalinization was greater in this series than in the non-injected mice and the number of mice showing noticeable hyaline changes was increased. In vagina and cervix we often found a rather marked hyalinization, especially of the muscle and connective tissue situated between the cervix and folds, but occasionally also of other parts of the muscle tissue. In addition, hyaline developed around some arteries. In a number of instances, however, the formation of fibrous-hyaline tissue was only very moderate in cervix and vagina. In the uterus the connective tissue was either fibrillar-cellular or fibrous-hyaline; often these changes were unequally advanced in different parts of the uterine wall. This fibrous-hyaline material extended in certain cases, also, into the muscular layer of the uterus; furthermore, hyaline rings of variable denseness were seen around some of the glands. More commonly the increase in the amount of intercellular hyaline substance affected the intercellular spaces, but sometimes distinct clumps of hyaline substance were visible into which the surrounding connective tissue penetrated. The density of this more or less homogeneous substance varied greatly in different animals; while in some it was loose in consistency, in others it was dense and some of the rings around the glands or the strands of hyaline beneath the surface epithelium were glossy in appearance.

III. The Stroma in Mice Receiving Larger Amounts of Estrogen

In mice receiving injections of 50 rat units of estrogen, in the large majority of cases daily, there is a further advance in the hyalinization of the vagina-cervix-uterus tract; but again considerable variations occur in individual mice and it is the uterus, as a rule, which is the better test organ for measuring in this respect the degree of effectiveness of estrogen injections. These variations may extend from a very scanty deposit of hyaline in the vagina, cervix and uterus, leading merely to a slight increase in intercellular hyaline, to a condition which approaches the very marked hyalinization observed in mice injected with 100 rat units of estrogen in oil weekly, although this latter effect is not, as a rule, attained. On the whole, in this group of mice we have still in the majority of cases to deal with an increase of intercellular hyaline in the stroma and an occasional extension of hyaline into the muscle of vagina, cervix and uterus; it is especially the connective tissue and muscle between the folds of vagina and cervix which may become markedly hyalinized. Furthermore in a number of instances some of the glands in the uterus or uterine cervix are found to be surrounded by a sheath of hyaline substance, which in some mice may be quite dense and glossy. Also around the arteries we may find some increase in hyaline material in various places,

particularly in the area between the two muscle layers; but neither in vagina nor uterus do we find, ordinarily, so marked a deposit of hyaline as around the arteries in the mammary gland.

The number of mice affected with hyalinization processes in the stroma of the genital tract, as well as the average degree of hyalinization, is greater in this group than in the two preceding groups. The most marked hyalinization was probably observed in an eighteen-month-old mouse which had been injected with estrogen for sixteen months. In this animal hyaline material of dense consistency was deposited in the vagina. There were still a number of connective-tissue cells imbedded in the hyaline, although some of these cells are probably always destroyed if this deposit becomes very dense. Between cervix and folds the muscle tissue had undergone marked hyalinization. There was hyaline material also in the lower part of the cervix but this diminished in amount in the upper cervix in the direction of the uterus. In the stroma of the uterus some clumps of dense hyaline tissue were found in certain areas and very dense hyaline rings had developed around some glands. The surrounding connective-tissue cells and blood vessels grew into the hyaline material and in places organized a part of it. The conditions found in the uterus in this case approached those seen in the group treated with 100 rat units of estrogen.

A peculiar condition, which will be described more fully in connection with the mice injected with 100 rat units, may be added to the hyalinization process in the genital tract. This condition occurs especially in the upper portion of the vagina and is usually most pronounced in the zone lying between the inner wall of the folds and the cervix. The connective tissue and muscle appear rarefied, almost as though they were perforated by a large number of small holes. As to the nature of this change, which appeared only exceptionally in the group of mice injected with 50 rat units, it is most probable that we have to deal with a mixture of hyaline and fluid material, due to solution processes which occur in the former.

In mice injected with 100 or more rat units of estrogen in oil once or twice a week, or with combinations of 100 rat units of estrogen in oil and 50 rat units of estrogen in water, the full development of tissue hyalinization is reached. Although in this group also some variations are found, yet, as a rule, the formation and distribution of hyaline substance in the vagina-cervix-uterus tract are very characteristic. The full development of hyalinization usually begins in the upper part of the cervix, especially in the uterine cervix, and from there the process extends deeper into the wall towards the peritoneal side; eventually this area may become markedly affected throughout its length. In the lower part of the cervix, also, there may be a deposit of hyaline, especially in the deeper part of the connective tissue and in the muscle tissue adjoining it. Furthermore, at the top of the folds some dense hyaline material is frequently observed. The typical finding in the lower part of the cervix, however, and especially in the connective tissue and muscle between the inner fold and cervix, and often in the deeper tissues of the vagina, is a state of rarefaction. As mentioned above, this condition is presumably due to a deposit of a mixture of hyaline material and edematous fluid as a result of solution processes in the hyaline substance. This rarefied material is commonly

FIG. 1. UTERUS OF C_3H FEMALE OVARIECTOMIZED AT TWO MONTHS; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR FOUR MONTHS AND SEVEN DAYS; KILLED AT SIX MONTHS AND SEVENTEEN DAYS

A. Hyaline material. *B*. Epithelioid cells. *C*. Gland. *D*. Uterine epithelium.

found in the deeper tissues of the vaginal wall; directly beneath the epithelium a connected layer of hyaline with a denser or looser consistency predominates. Certain variations may be observed, however, in the amount of hyaline present in the vagina, and in some instances the deeper parts may also be densely hyaline. Likewise, in the lower and intermediate portions of the cervix hyaline material of varying consistency may almost entirely replace the muscle tissue, and strands of dense hyaline tissue may be found around folds and directly beneath the cervical epithelium.

The uterus is usually very strongly affected and hyaline may fill the greater part of the connective tissue between the surface epithelium and muscle tissue. Frequently dense hyaline strands invade the muscle tissue, leading to its destruction and replacement by hyaline. In some cases a layer of hyaline forms beneath the surface epithelium, or dense hyaline rings may enclose some of the glands. Quite frequently, however, a layer of cellular-fibrillar connective

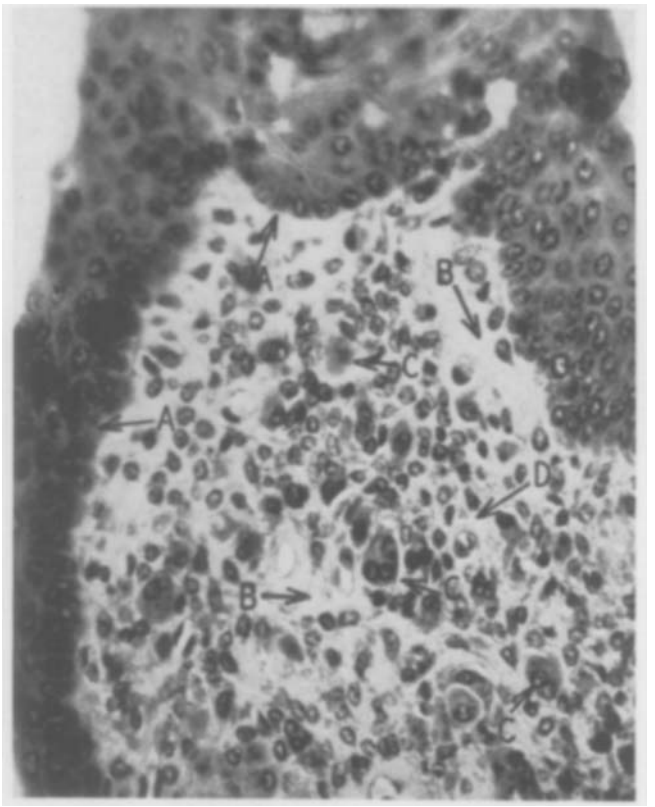

FIG. 2. VAGINA OF C_3H FEMALE OVARIECTOMIZED AT TWO MONTHS; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR FOUR MONTHS AND SEVEN DAYS; KILLED AT SIX MONTHS AND SEVENTEEN DAYS

A. Vaginal epithelium. *B*. Remnants of hyaline material. *C*. Small giant cells. *D*. Connective tissue which has organized the hyaline material.

tissue pushes its way directly beneath the epithelium and separates it from the hyaline which is deposited in the deeper areas of the uterine wall. It seems that the connective-tissue cells move preferably in the area between the epithelium and the adjoining hyaline material. Hyaline may also surround the arteries, especially between the two muscle layers, but on the whole, as we have stated above, a densely hyaline sheath around the arteries is not usual.

This process of hyalinization, especially in the cervix and uterus, and to some extent also in the vagina, is counteracted by invasion by connective tissue. This may occur in the deeper tissues as well as beneath the epithelium, whence the cellular connective tissue may grow downward into the hyaline substance. In this way there may take place in many areas a substitution and organization of the hyaline material by connective tissue, in which dilated capillaries may also be visible. Isolated patches of hyaline may then be found in the fibrillar connective tissue, or in the end only small remnants of hyaline material may be seen here and there. It is probable, however, that this process of organization by connective tissue may again be interrupted by new deposits of hyaline and in the end the latter process seems to prevail over the ingrowth

FIG. 3. UTERUS OF C_3H FEMALE OVARIECTOMIZED AT TWO MONTHS; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR FOUR MONTHS AND SEVEN DAYS; KILLED AT SIX MONTHS AND SEVENTEEN DAYS [2]

A. Uterine gland lined with cylindrical epithelium. *B*. Cylindrical epithelium of uterus. *C*. Hyaline material. *D*. Thin subepithelial layer of connective tissue separating hyaline tissue and uterine epithelium. *E*. Slightly dilated gland filled with colloid material.

of connective tissue. This appears likely in view of the fact that in animals eighteen months old or more, which had been injected with estrogen for the greater part of their lives, dense hyalinization of vagina, cervix and uterus seemed to predominate.

There is a second process which in many cases accompanies the invasion and organization of hyaline substance by connective tissue, namely a formation, at the margin of the hyaline material, of epithelioid and of small giant cells possessing more than one nucleus. These cells also seem to invade and destroy the hyaline material and occasionally some of the latter is apparently found within the cytoplasm of the giant cells. In only a certain number of cases, however, where connective tissue invades the hyaline, do epithelioid and giant cells develop; usually they are absent. As a rule, the epithelioid cells are seen alone; giant cells are more rare. We have to deal in these instances

[2] We are indebted to Dr. H. A. McCordock for this photomicrograph.

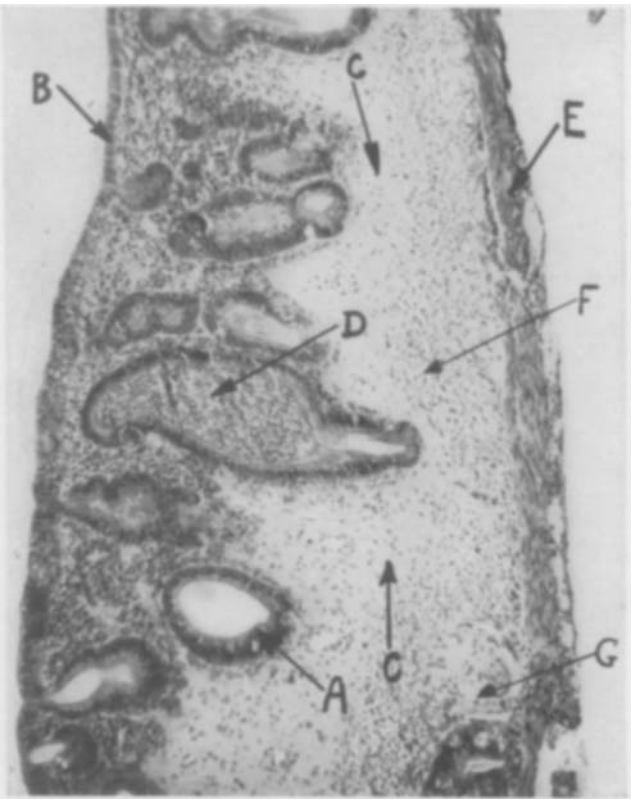

FIG. 4. UTERUS OF C_3H FEMALE OVARIECTOMIZED AT TWO MONTHS; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR SIX AND ONE-HALF MONTHS; KILLED AT EIGHT MONTHS AND TWENTY-FIVE DAYS

A. Uterine gland lined with cylindrical epithelium. *B*. Cylindrical epithelium of uterus. *C*. Hyaline material. *D*. A cystically dilated gland. *E*. Outer muscle layer of uterus. *F*. Inner muscle layer partly hyalinized. *G*. Hyaline around artery between two muscle layers.

with foreign body reactions, the hyaline substance deposited in the connective tissue or muscle acting not unlike bits of coagulated blood serum or agar experimentally introduced. The formation of epithelioid and giant cells may be observed in the vagina and uterus in young and middle-aged mice, but it is apparently more frequent in the former; we have not so far observed it in mice older than twelve months. This reaction may appear as early as one month after the beginning of the estrogen injections; that is, after four weekly injections. In general, the formation of epithelioid and giant cells is associated with an invasive activity of the connective tissue which leads to an organization of the hyaline substance; furthermore, it seems to occur especially in cases in which the consistency of the hyaline is denser.

As to the origin of the hyaline substance, a rarefaction of the connective tissue may be observed in certain parts of the upper vagina near the folds, and in the tissue between folds and cervix, as early as two weeks after the beginning of the experiment, when only two injections have been given. In the uterus, also, the connective-tissue fibrils may in places appear somewhat separated, perhaps by edematous fluid. There may be some intercellular hyaline

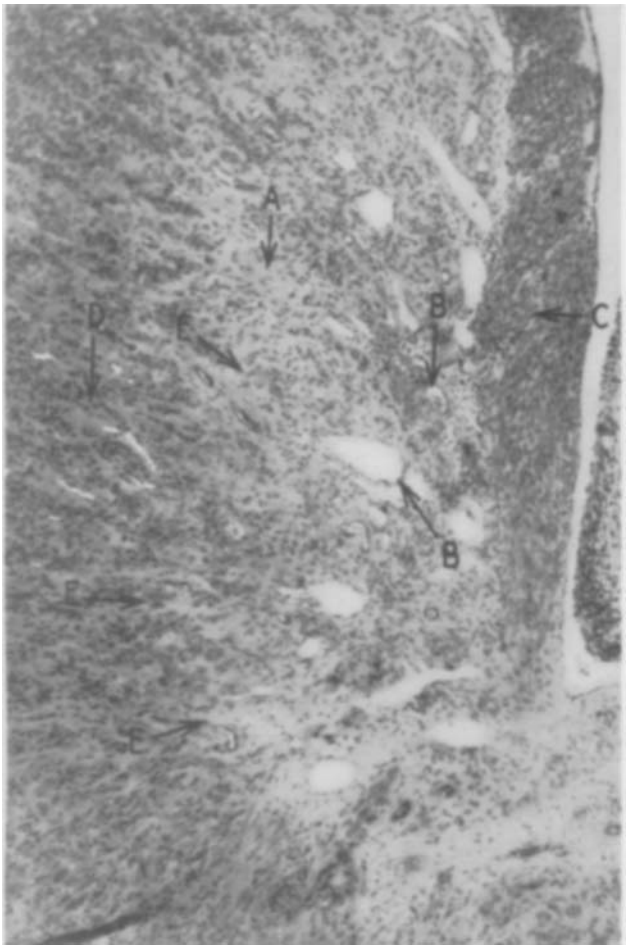

FIG. 5. UTERUS OF C_3H FEMALE OVARIECTOMIZED AT TWO MONTHS; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR SIX AND ONE-HALF MONTHS; KILLED AT EIGHT MONTHS AND TWENTY-FIVE DAYS

A. Hyaline material between two muscle layers. *B*. Capillaries. *C*. Outer muscle layer of uterus. *D*. Remnants of inner muscle layer of uterus. *E*. Hyaline material invading and replacing part of inner muscle layer.

in the vagina, within the muscle tissue of the cervix, and in the deeper parts of the uterine mucosa near the muscle. The first changes consist very likely in the transudation of fluid from the vessels into the connective tissue. After four to six weeks the rarefied condition of the stroma, described above, is observed. There may be noticed at this time in the vagina a slight increase in hyaline between the cells or directly beneath the epithelium; in the upper part of the cervix there may be a certain increase in intercellular hyaline material. In the uterus the stroma seems edematous in places; but here, too, some hyaline deposits are found between the cells of the mucosa, around the glands, and in the inner muscle layer. The density of the hyaline substance varies in different places. At this stage the connective tissue may begin to penetrate into the hyaline deposit and the first epithelioid cells may appear.

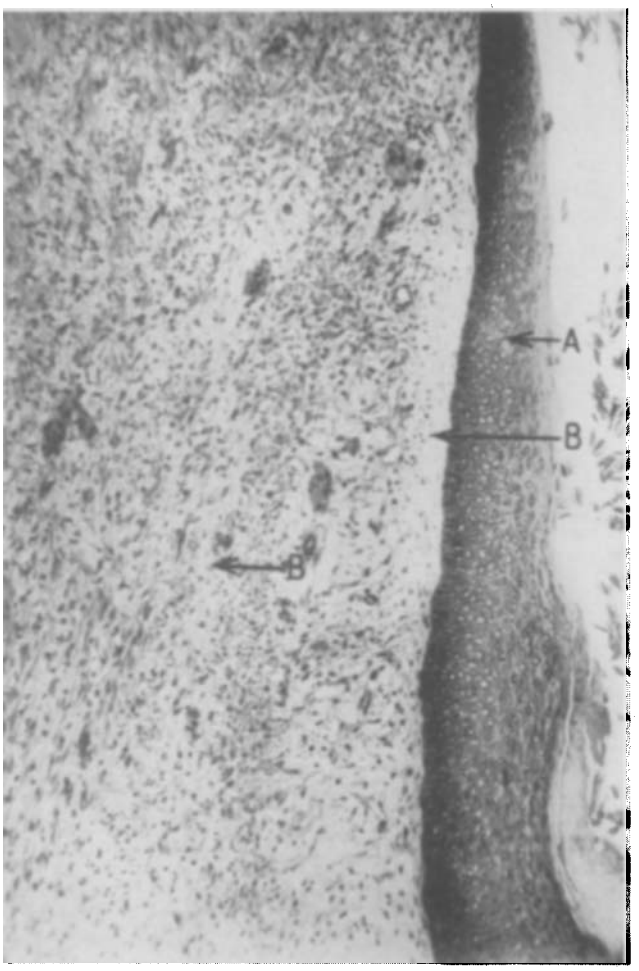

FIG. 6. VAGINA OF C_3H FEMALE OVARIECTOMIZED AT ONE MONTH; INJECTED WITH 150 RAT UNITS OF ESTROGEN WEEKLY FOR TWO MONTHS; KILLED AT THREE MONTHS

A. Squamous epithelium lining vaginal fold. *B*. Rarefied (vacuolated) connective tissue and muscle tissue separating vaginal fold and cervix.

It seems, then, that at a very early stage after the beginning of the injections of effective doses of estrogen, a liquid substance which separates the connective-tissue elements makes its appearance, and that this represents one of the earliest changes induced by the hormone. It may be accompanied, or soon followed, by the deposit of a hyaline substance which occurs first between the connective-tissue cells, but may extend also to the muscle fibers. With increase in the amount and density of this hyaline substance, connective-tissue elements become more and more separated and may be destroyed. At last the hyaline, if it has reached a certain degree of density and has become poor in cells, may act as a foreign body and initiate secondary reactions on the part of certain host tissues and cells. These may be followed by new deposits of the same material.

IV. Stroma in Mice Receiving Estrogen in Combination with Other Hormones

In mice injected with combinations of estrogen and acid extract of cattle anterior pituitary gland the results are similar to those obtained with the same amounts of estrogen alone—between 1 and 30 rats units at each injection. In mice below the age of six months the hyaline substance in the vagina and cervix is usually soft and moderate in amount. In the uterus the stroma is cellular-fibrillar, or a very little intercellular hyaline is present, which may occasionally extend in slight amounts into the muscle tissue. In mice between the ages of six and eighteen months conditions are similar, but in several instances some hyaline was found beneath the epithelium of the surface and of the glands in the uterus. In mice above the age of eighteen months we found in several cases a more dense hyalinization in vagina and cervix and also in the uterus, and it seemed that connective tissue penetrated the hyaline in places. However, in other instances the stroma was mostly of the usual fibrillar-cellular type. There existed thus considerable individual variations. The state of the stroma depended also on the amount of estrogen injected. Extract of cattle anterior pituitary gland alone was apparently without effect.

Combinations of various amounts of estrogen and lutein preparations acted like estrogen alone; the results depended on the efficiency of the estrogen preparation used, on the length of time during which it acted, and on certain variations in individual mice. Lutein preparations alone did not exert a permanent effect on the stroma.

Discussion

Our experiments show that injections of estrogen in mice produce a series of changes in the stroma of the vagina, cervix and uterus, which differ somewhat in different areas but consist essentially in the deposit of a hyaline substance of variable density in the connective tissue and unstriated muscle tissue. This effect is most marked in the cervix and uterus; it occurs to a less extent in the vagina. If the estrogen effect becomes very strong a less dense material, in which solution processes seem to take place, is apparently deposited in the stroma, especially in the deeper parts of the vagina and between the cervix and folds, so that the tissue assumes a rarefied structure. The exact mechanism underlying this process of rarefaction needs further investigation.

There exists an approximate proportionality between the doses of estrogen given and the continuity of its action and the amount and density of the hyaline substance which is deposited. Extreme reactions are obtained if injections of 100 or more rat units of estrogen in oil are injected weekly over a certain length of time. Under such conditions the deposit of hyaline, which progressively becomes more and more devoid of connective-tissue cells and blood vessels, is so marked that the material acts like a foreign body and induces characteristic reactions on the part of the host tissues. In normal, non-injected mice, also, there are indications that with increasing age an increase in the formation of fibrous-hyaline substance takes place. This begins at a very early stage in life and it again becomes especially noticeable in mice past the age of sexual functioning. During the period of sexual activity these

changes are not so definite. However, the great variability in the development of fibrous-hyaline material in the stroma in different individuals makes further studies concerning the stroma changes in normal mice advisable.

Notwithstanding these limitations in our conclusions so far as normal mice are concerned, there remains little doubt that in respect to the intensity and character of the stroma changes a connected and progressive series exists, beginning with the conditions found in normal animals and extending through those in animals injected with smaller and finally with larger, more effective doses of estrogen. The last group exhibited in a very much exaggerated form conditions which are present in a rudimentary fashion in certain normal mice and in mice injected with relatively small doses of estrogen.

As to the origin of the hyaline substance deposited in the stroma, there are some indications that it may perhaps be derived, at least in part, from a constituent of the blood plasma, because, as stated, certain transudative changes are among the earliest observed. Although the cooperation of cells in its production could not be observed, the possibility that in some way they may be involved in this process cannot be excluded. At first the substance seems to be deposited between the connective-tissue cells, but as it increases in amount and density these cells seem to undergo injury. Especially characteristic is the situation of this material in the form of hyaline rings around glands and in the form of a dense hyaline layer directly beneath the surface epithelium of vagina and cervix; furthermore, it penetrates into and may largely replace parts of the muscle tissue. While it may be found also around blood vessels, such deposits are less conspicuous than in other organs in mice, such as the mammary gland, and in the uterus and elsewhere in certain other species. There is a tendency for the hyaline substance to form sheaths around various organs and it is more prominent at the border separating different tissues and organs. This may perhaps be due to the fact that in these localities it is best protected against solution processes which seem to affect it under certain conditions.

In its appearance and in the foreign body reactions which it initiates this substance somewhat resembles amyloid, which is readily produced in mice in various groups. The application of stains differentiating amyloid from other hyaline material, however, gave negative results.

We have seen that epithelium and stroma in various parts of the vagina-cervix-uterus tract react somewhat differently to injections of estrogen. Differences in these tissues are found also as far as the reaction of the stroma to a diminution in the supply of estrogen is concerned. Such a diminution may be produced through ovariectomy. While this latter procedure seems to favor the assumption of a fibrous-hyaline character by the stroma of the vagina, in the uterus it leads to the production of a stroma consisting of densely packed cells in which small nuclei are mainly visible, while the fibrillar intercellular substance is quite inconspicuous; it is only in the deeper layers of the stroma near the inner muscle layer that the fibrillar or hyaline intercellular substance becomes more prominent. We may tentatively assume that ovariectomy may exert two different effects: (1) due to withdrawal of the stimulating action of estrogen, it leads to an atrophy of all the tissues of the sex tract; (2) on account of the inactivity of the stroma which it induces, it may be followed by

the development or deposit of a fibrous-hyaline intercellular substance. The former process predominates in the uterus, where it results in a decrease in the intercellular tissue elements and a diminution in the size of the cells. The latter process predominates in the vagina, although atrophic changes may take place in this organ as well. Also, the epithelium as a rule becomes inactive and undergoes atrophy in the three organs of the sex tract. In a number of cases, however, we observed the formation of squamous epithelium in vagina and cervix of apparently completely ovariectomized mice, in which at autopsy no remnant of ovarian tissue could be found.

We see then that a hormone, estrogen, has two apparently opposite effects on the stroma of the vagina-cervix-uterus tract. (1) The increased activity of the epithelial structures and perhaps also variations in the condition of the blood vessels, which take place under the influence of estrogen, may cause an increased activity in the stroma, leading to an increase in the size and proliferation of the cells and to a decrease in the amount of intercellular substance. Such an effect is very pronounced, for instance, in the mammary gland of an immature guinea-pig under the influence of anterior pituitary hormones, provided they cause a full maturation of ovarian follicles and therefore a discharge of estrogen. (2) Under the influence of large doses of estrogen administered over long periods of time an increase in the amount of intercellular fibrous-hyaline substances occurs; the larger the amount of active estrogen, the greater is this increase. This change in the stroma will interfere with the exchange of fluids between parenchyma and lymph and blood vessels and will therefore have injurious effects on the nourishment and activity of tissues and organs, tending to bring about some of the conditions which are characteristic of old age.

The deposits of hyaline substance exert a particularly injurious influence on growing tissues. In the mammary gland of the mouse hyaline tissue tends to cause an atrophy, and in certain cases a disappearance, of tubules and acini; it seems also to interfere somewhat with the process of secretion. It might be, concluded, therefore, that estrogen, by producing hyalinization, may indirectly inhibit to a certain extent the changes which lead from normal to precancerous tissues, although on account of its direct stimulating action on the epithelial elements of the mammary gland it tends to promote such a transformation, a result which in the end predominates. Such a conclusion, however, would hold good only if injections of estrogen should exert similar effects on the stroma of the mammary gland as they do on the stroma of uterus and cervix, and this is a question which needs further investigation.

On the other hand, the presence of densely hyaline material calls forth in cervix and uterus of mice a long-continued, increased proliferative activity of the surrounding connective tissue cells and it is possible that processes of this kind were responsible, perhaps in association with other factors, for the development of a sarcoma in cervix and vagina which we observed in a mouse injected subcutaneously over a long period of time with large doses of estrogen.

SUMMARY

In normal mice an increase in the amount of collagen in the stroma of vagina, cervix, and uterus takes place with increasing age. This increase

begins in the first few months of life. It appears to make little advance throughout the sexually active interval, but it progresses again during the later period of life. In individual animals certain variations in the intensity of these changes seem to occur but, on the whole, the differences found at different age periods are relatively not very great. The amount of hyaline substance developing with increasing age around the arteries in organs of the sexual tract is much less than in the mammary gland in mice. Further studies will be necessary to evaluate with greater accuracy finer differences which develop normally with advancing age.

Injections of estrogen over long periods of the life of the mouse increase the amount of fibrous-hyaline material deposited in the stroma. This increase is the greater, the greater the amount of effective estrogen administered and the longer the period of its administration. With the largest doses of estrogen, 100 or more rat units dissolved in oil, injected weekly, the hyaline substance may be deposited in the form of clumps or strands, which act as foreign bodies and elicit typical foreign body reactions, consisting in the formation of epithelioid and giant cells and the ingrowth of connective tissue; but this organization process, as a rule, seems to be followed and counteracted by new deposits of hyaline substance throughout the period of injections. Besides the deposit of hyaline substance, a rarefaction of the stroma becomes noticeable in certain places, such as the deeper parts of vagina and the area between the cervix and folds. This latter process is due perhaps to transudation of fluid from the blood vessels and to subsequent solution of some of the hyaline material. In other areas, on the other hand, especially in the upper part of cervix and uterus, hyalinization in connective tissue and unstriated muscle, and to a more limited extent also around arteries, predominates. Certain variations occur, however, in the distribution of this substance in individual animals.

A hormone, estrogen, may therefore affect the stroma of various organs in two opposite directions. (1) By inducing growth processes in the epithelial structures, and perhaps also by its effects on the circulation, it may cause a diminution in the amount of fibrous-hyaline material in certain organs, and there are indications that it may exert these effects in the vagina, cervix and uterus. (2) But if large doses of this hormone are administered over long periods of time, the opposite effect may be obtained, namely, a very marked increase in the amount of fibrous-hyaline material in the stroma. In this way it is therefore possible to initiate and to intensify in certain organs changes similar to those occurring in old age. An increase in the amount and density of the fibrous-hyaline substance in the stroma tends to exert pressure on functionally active and proliferating tissues and may thus cause interference with food and oxygen supply, which in the end may, in certain instances, lead to the destruction of the more sensitive structures. On the other hand, it is possible that constant stimulation of the connective tissue by a foreign body such as dense hyaline, perhaps in cooperation with other factors, may in some cases lead to the production of sarcoma.

References

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