PIGMENTATION OF THE SEX GLANDS IN VITAMIN E DEFICIENT RATS*

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Rats deprived of vitamin E for several months after weaning exhibit a faint brownish pigmentation of the uterus which gradually increases to chocolate-brown color as the deficiency progresses, and is associated with the gradual accumulation of coarse brownish granules within the smooth-muscle cells of the myometrium. A similar but somewhat less pronounced change occurs in the smooth musculature of the cervix, vagina, oviducts, seminal vesicle, and ureter, and also in the skeletal musculature (Martin and Moore,[13, 14] Barrie,[1] Hessler,[10] and Demole[3]). The smooth musculature of the digestive, respiratory, and vascular systems, and of the urinary bladder is said to be immune. Although a brownish discoloration of the testis and abdominal lymph nodes has been reported (Martin and Moore[14]), no histological observations have been made and no explanation offered for the deposition of pigment in organs in which smooth-muscle cells are limited to those of associated blood vessels.

The observations cited above have been generally confirmed and considerably extended by studies in this laboratory, as discussed briefly in a recent review (Mason[17]) and presented in detail elsewhere (Mason and Emmel[18]). By appropriate staining methods we have demonstrated that the granular changes in smooth-muscle cells are invariably associated with the appearance of connective tissue macrophages containing large numbers of similar granules.† These phagocytes are especially abundant in the myometrium and undoubtedly contribute much to the discoloration of the organ. Similar but smaller phagocytic cells also occur in association with the necrosis of skeletal muscle, and with that of cardiac muscle which we have noted after prolonged states of E deficiency.

The presence of pigment-laden phagocytes in the lymph nodes and spleen and of pigment globules in the Kupffer cells of the liver

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† Although previous investigators have referred to the pigment observed in muscle cells as "granules," we prefer to use the term "globules" since this describes more accurately the appearance of this pigment as it occurs in the tissue macrophages and elsewhere.

represents a wide dispersal, via the lymphatic and vascular channels, of this relatively inert material arising chiefly as a product of deranged metabolism and necrosis in the musculature of the body. In histological preparations the globules within smooth-muscle cells and macrophages everywhere have a specific affinity for basic fuchsin and, to some extent, for iron hematoxylin. Their poor delineation by hematoxylin and eosin and by other routine histological stains explains why the phagocytic cells escaped the attention of earlier investigators.

These pigment globules appear to be liberated from smooth-muscle cells with little or no necrosis occurring, but from skeletal and cardiac muscle only during necrosis and death of the fibers. The evidence available suggests that this pigment material represents either (1) a normal metabolite which is readily utilized or degraded by normal cells but not by cells deprived of vitamin E, or (2) an abnormal metabolite which the cells are unable to utilize or break down to any appreciable extent. Either explanation must have as its basis some specific biochemical lesion, perhaps the dysfunction of some cellular enzyme system in which the vitamin plays a vital rôle. The fact that this pigment material shows little or no evidence of breakdown after its retention for prolonged periods by the tissue macrophages favors the second alternative.

The conclusions reached concerning the muscle changes just discussed have necessitated a different explanation for a striking accumulation of pigment phagocytes which we encountered several years ago in the ovaries and testes of our E deficient rats—an observation which greatly increased the complexity of the histological picture and the difficulties in ascertaining the primary source of the pigment material. Obviously, this material could not have a myogenous origin, either directly or indirectly by way of lymphatics from other tissues. Repeated study of older tissues by other technics, supplemented by much new material, has provided an explanation for the pigmentation of the sex glands which differs from, but is quite compatible with, the conclusion reached concerning pigment accumulations elsewhere. The present report presents the evidence obtained in support of this interpretation.

Material and methods

The ovaries and testes of 60 female and 30 male rats, reared from weaning on E deficient diets and killed at various intervals between the 40th and 600th days of life, have been examined by a variety of histological

technics. Thirty control rats of both sexes were treated in the same manner, but were given adequate oral supplements of vitamin E. Best results were obtained by fixation in Zenker-acetic or Zenker-formol and the staining of paraffin sections (5 to 7 microns thick) with iron hematoxylin or Ehrlich's hematoxylin, counterstained with Mallory's basic fuchsin. For studying simultaneously the distribution of iron and pigment material the Turnbull blue method (Prussian-blue reaction) demonstrating inorganic iron, followed by the Mallory's basic fuchsin counterstain, proved especially useful. Other special methods will be referred to in connection with the experimental observations.

Ovaries of normal rats

In the ovaries of adult rats and of other small rodents with brief estrous cycles the amount of cellular degeneration involved in the processes of follicular atresia and corpus luteum regression, per unit of tissue, has no parallel in any other organ or tissue in the body. Although this rapid turnover of cells in the ovary has long been accepted, little effort has been made to evaluate it or to study in detail the mechanisms whereby the products of this cell degeneration are removed.

Tissue macrophages are abundant in the normal ovary and undoubtedly play an important rôle in this process, as is demonstrated by vital staining of these cells (Borell,[2] Long and Evans[12]). In properly stained sections of fixed material they are found most frequently in the center of small atretic follicles, often surrounding early cleavage stages or degenerated fragments of the ovum; they also occur in corpora lutea showing advanced stages of regression (Fig. 7). Their finely granular and faintly acidophilic cytoplasm often appears much vacuolated and foam-like. After follicular atresia and luteal regression are complete, these phagocytes become incorporated in the interstitial stroma and in the loose connective tissue of the medulla where they remain until they succeed in entering the lymph sinusoids (Fig. 8). The ovaries of adult animals contain a relatively constant number of these cells, many of them greatly flattened and elongated by the pressure of growing follicles and large corpora lutea. In rats a year or more old the ovaries may show a moderate increase in number.

In preparations stained for iron by the Turnbull blue-basic fuchsin method these macrophages are readily identified because they frequently contain Prussian-blue granules and show a moderately strong affinity for basic fuchsin. The presence of iron in phagocytes

adjacent to areas of recent hemorrhage in corpora lutea is generally recognized, but little or no attention seems to have been given to its widespread occurrence in phagocytes of small atretic follicles which we find to be such a constant phenomenon in the ovaries of normal and E deficient rats. It is difficult to determine how much of this iron is derived from the degenerate ova, which often contain a few scattered blue granules, and how much is released from nucleoproteins in degeneration of the follicle cells; a hematogenous origin seems doubtful since the theca cells form a barrier to the capillaries surrounding such undeveloped follicles. Except for perhaps a slight increase in number of these macrophages in old animals, their relative abundance and their content of iron and of material staining with basic fuchsin show but little variation in normal adult rats. This suggests a relatively constant balance between the processes of cellular degeneration and the ability of the tissue macrophages to phagocytize the debris and remove it from the organ—a mechanism which exhibits a definite derangement in the E deficient rat, as discussed in subsequent sections.

Ovaries of vitamin E deficient rats

In ovaries of vitamin E deficient rats varying from 3 to 20 months of age the amount of iron present is also remarkably constant

PLATE I

FIG. 1. Ovary of control rat 4159, age 148 days, reared on E deficient diet supplemented with adequate vitamin E. The majority of macrophages contain both granules of Prussian blue and other globules showing affinity for basic fuchsin; a few show only one or the other type of material. These macrophages occur individually and in small groups, especially in the interstitial tissue, and appear as irregular black specks in the photomicrograph. Turnbull blue-basic fuchsin stain. ×45.

FIG. 2. Ovary of E deficient rat 4135, age 100 days. The total number and general staining reactions of the macrophages are essentially the same as found in normal adult rats of similar ages. The tendency seen here for these cells to concentrate in areas of follicular atresia and of luteal regression is also characteristic of normal rats. Turnbull blue-basic fuchsin stain. ×45.

FIG. 3. Ovary of E deficient rat 146, age 190 days, showing a distinct increase in macrophages throughout the interstitial tissue; many of these are being compressed and flattened by growing follicles. A relatively small number of these cells contain granules of Prussian blue, which is present to about the same extent as in control rats (cf. Fig. 1). Turnbull blue-basic fuchsin stain. ×45.

FIG. 4. Ovary of rat 3104b, E deficient to 224 days of age followed by 106 days of high vitamin E therapy. Note extensive accumulation of macrophages throughout the interstitial areas. Prussian blue granules are present in a few macrophages, and to the extent seen in normal ovaries. Binucleate and multinucleate macrophages, such as shown in Fig. 12, are relatively abundant. Accumulation of macrophages in this ovary is much more extensive than that seen in Fig. 3, but somewhat less than in the ovary of rat 3112 of similar age but not given vitamin E therapy (Fig. 5). Turnbull blue-basic fuchsin stain. ×45.

FIG. 5. Ovary from E deficient rat 3112b, age 360 days, showing advanced stage of pigment accumulation. Except for the small follicles (F) and the small blood vessels (B), all of the dark staining material represents accumulations of pigment-laden macrophages which occupy a large portion of the interstitial tissue and are also scattered throughout the medulla of the organ. Iron hematoxylin-basic fuchsin stain. ×45.

FIG. 6. Same ovary as in Fig. 5 but stained to bring out more brilliant contrast between the pigment-laden macrophages and the ovarian stroma. The intensely black areas represent irregular clusters of macrophages; most of these still retain their individuality, but some have coalesced and form binucleate and multinucleate giant cells such as are shown in Fig. 12. Ehrlich's hematoxylin-basic fuchsin stain. ×45.

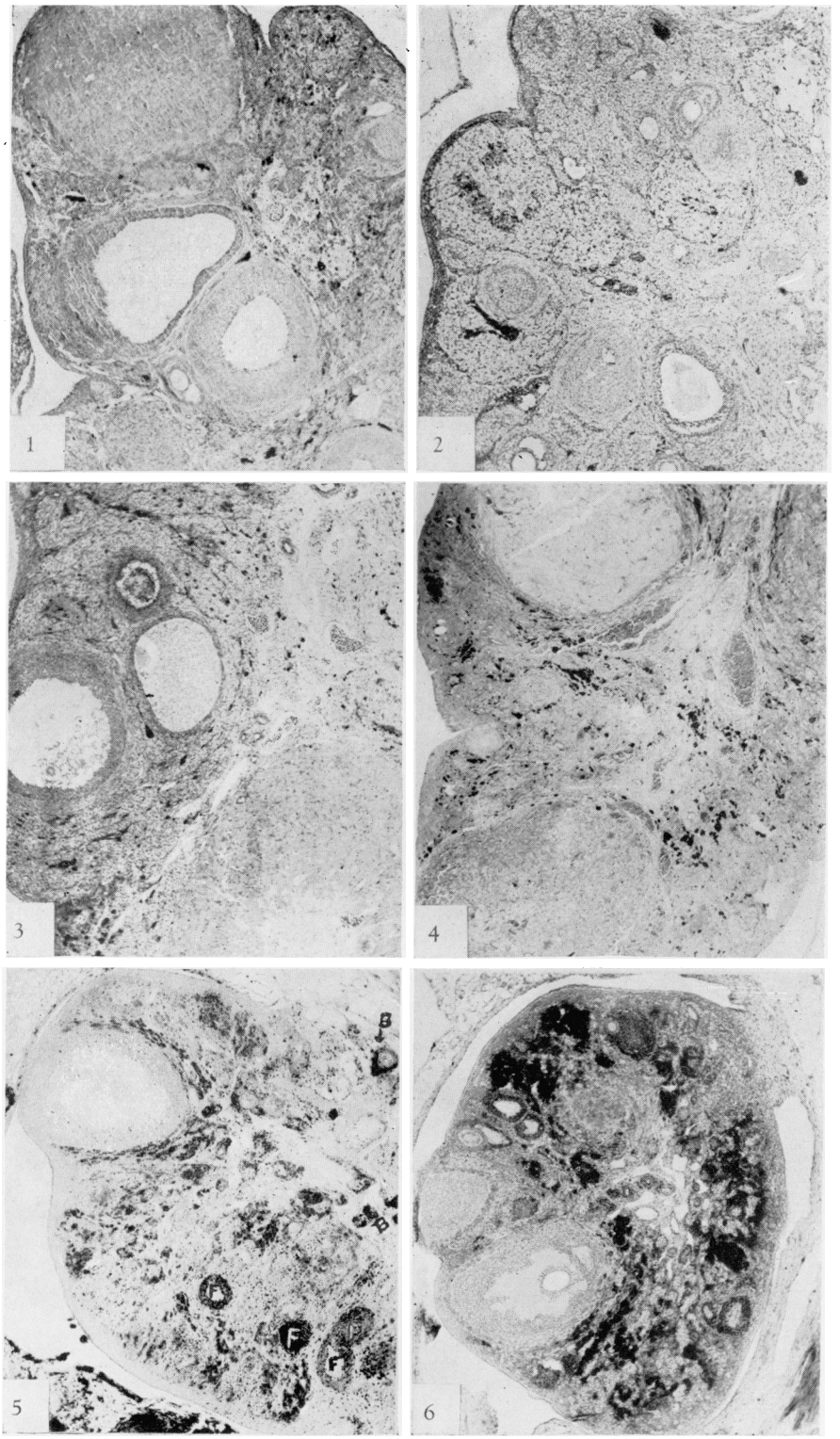
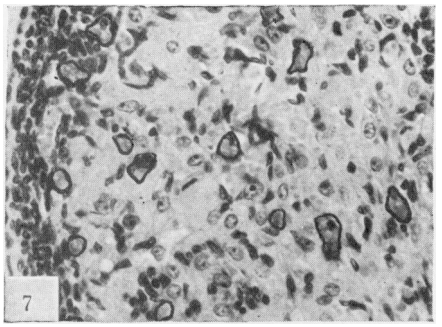
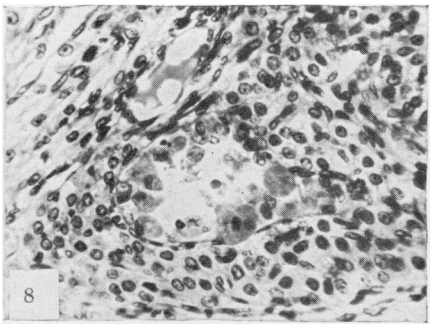
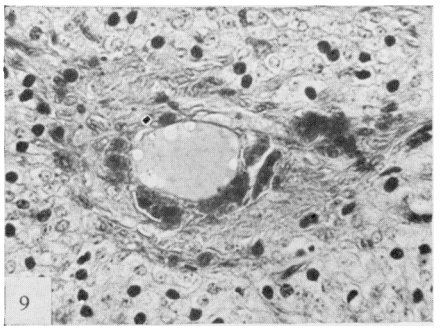
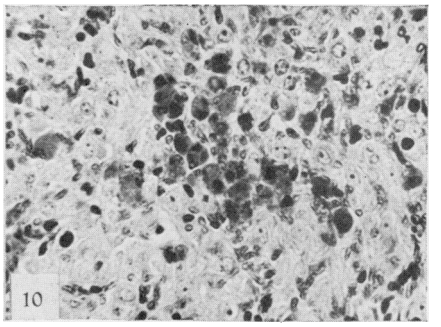


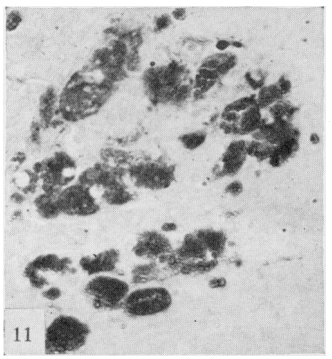
PLATE I

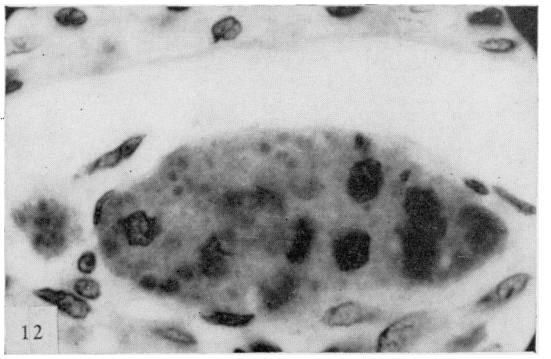


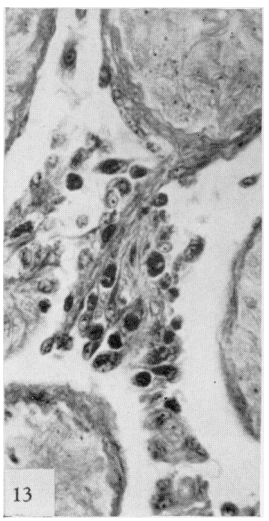


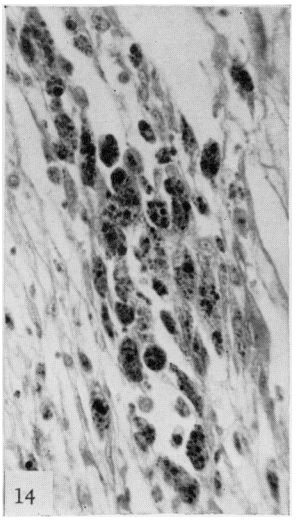












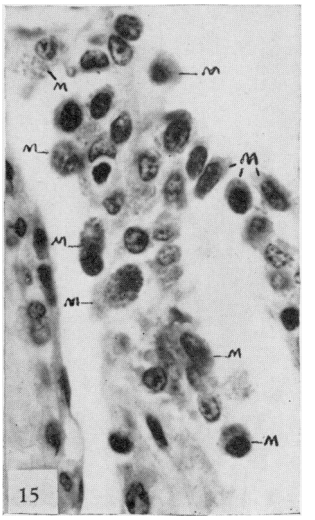


PLATE II

and compares favorably with that observed in control rats. On the other hand, there occurs a slow but progressive increase in the number of macrophages staining with basic fuchsin and perhaps a corresponding increase in their affinity for this dye.

In rats 100 days old these macrophages are no more abundant than in the ovaries of normal control rats (cf. Figs. 1 and 2). Several months later those present in the interstitial tissue are definitely increased in number (Fig. 3) and stain more intensely; those related to the follicles and corpora lutea are, and continue to be, about as numerous as before but they usually appear denser and more deeply stained (Figs. 9 and 10). As the deficiency progresses the macrophages gradually accumulate in the interstitial tissue and adjacent medulla so that, by the end of the first year of life, the stroma of the ovary is literally riddled with macrophages, chiefly in the form of irregular clusters and larger clumps which stain intensely with fuchsin (Figs. 5 and 6).

The sequence of events involves four features of particular significance: (1) the necrotic ova, granulosa cells, and lutein cells, as

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PLATE II

FIG. 7. Corpus luteum in advanced stage of regression from control rat 4134, age 100 days. The cells outlined on the photomicrograph in India ink are macrophages which are readily distinguished from the surrounding lutein cells in the histological preparation but are not sharply differentiated in the photomicrograph. Ehrlich's hematoxylin-basic fuchsin stain. ×287.

FIG. 8. A group of typical macrophages within a lymph channel of the interstitial tissue, in the same preparation from which Fig. 7 was taken. One large cell at lower center is in the process of entering the sinusoid, as verified by examination of adjacent serial sections. Ehrlich's hematoxylin-basic fuchsin stain. ×287.

FIG. 9. Atretic follicle from ovary of E deficient rat shown in Fig. 3. Eight pigment-laden macrophages surrounding the cavity of the follicle, and a small clump of similar cells at the right, are stained deeply with fuchsin. Iron hematoxylin-basic fuchsin stain. ×287.

FIG. 10. Similar macrophages in the center of a corpus luteum in advanced stage of regression, from same ovary as Fig. 9. Iron hematoxylin-basic fuchsin stain. ×287.

FIG. 11. Cluster of macrophages in the interstitial tissue of ovary shown in Fig. 4, from rat given vitamin E therapy. The granular and vacuolated appearance and marked enlargement of these cells, in comparison with those from younger animals (Figs. 9 and 10), are characteristic features after prolonged deficiency, whether followed by E therapy or not. A small amount of Prussian blue is present in the uppermost group of cells; otherwise all of the dark-staining material reacts specifically with basic fuchsin only. Turnbull blue-basic fuchsin stain. ×287.

FIG. 12. Giant cell formed by fusion of macrophages whose wrinkled nuclei (portions of ten nuclei are demonstrable in this section) and coarse irregular globules of pigment material are readily seen. A fragment of what proves to be a binucleate cell lies at the left of the giant cell. Both are immediately external to a lymph sinusoid (above). From ovary of rat 3259, given vitamin E therapy for 120 days after 268 days of E deficiency. Ehrlich's hematoxylin-basic fuchsin stain. ×1000.

FIG. 13. Intertubular area from testis of E deficient rat 4150, age 380 days, showing numerous pigment-laden macrophages which are readily distinguishable from other cells of the interstitial tissue by their intense black appearance due to their marked affinity for basic fuchsin. Iron hematoxylin-basic fuchsin stain. ×287.

FIG. 14. A cluster of macrophages located in the "tunica vasculosa testis," the loose connective tissue just internal to the capsule, from the same testis as Fig. 12. These cells are often binucleate, sometimes multinucleate, which accounts for their larger size in comparison with those in the interstitial areas (Fig. 13). The staining character of their cytoplasm and contained globules also is suggestive of physiological senility. Iron hematoxylin-basic fuchsin stain. ×287.

FIG. 15. Interstitial area from the same testis as Fig. 12, but more highly magnified to show the cytoplasmic globules of the macrophages, indicated by M. Only in a few cells are these globules in sharp focus. Ehrlich's hematoxylin-basic fuchsin stain. ×626.

well as fragments of such cells frequently encountered, are essentially normal in number but show an increased density and affinity for basic fuchsin; (2) the macrophages immediately concerned in their removal are likewise no more abundant than usual, but are less vacuolated and more deeply stained; their ability to phagocytize Trypan blue appears to be unimpaired, excepting in so far as this function is depressed by the gradual accumulation of pigment globules in their cytoplasm; (3) leukocytes are present in no greater numbers than in control rats; (4) it is impossible to state with certainty whether the ovarian pigment represents an increment of an intermediate breakdown product released to a lesser degree in normal ovaries in the process of follicular atresia and luteal regression, or a definitely abnormal metabolite accumulating in the ovarian cells and released with, or perhaps without, degeneration of these cells in the above mentioned process. Although the latter explanation would be in closer accord with our concept of the mode of origin of this pigment in muscle tissues,[18] the absence of accumulations of this pigment in functionally active granulosa and luteal cells, the occurrence in normal ovaries of a pigment indistinguishable from that encountered in deficient rats, and lack of evidence that follicular atresia and luteal regression are accentuated by E deficiency, tend to favor the first alternative.

As deficiency advances this process continues at a relatively constant rate. After from 18 to 20 months from one-half to two-thirds of the interstitial tissue is composed of large pigment-laden macrophages. Yet the physiological function of the ovary and the associated morphological changes (follicular growth, ovulation, corpus luteum formation), even in rats 500 to 600 days old, are as normal as those observed in control rats of similar age. In these respects the uterus of the E deficient rat reveals a striking parallel. Both phenomena emphasize the specific relationship of vitamin E to some metabolic derangement in cells which is compatible with continued function of the organ and is reflected chiefly in a generalized embarrassment of tissue macrophages and, eventually, of other components of the reticulo-endothelial system.

In teased preparations from fresh ovaries of rats deprived of vitamin E for a year or more and injected with Trypan blue, the clusters of macrophages can easily be dissected out as large brownish, sometimes orange-brown, masses. Neither the individual macrophages packed with pigment globules, nor the interstitial cells which

serve to unite them in irregular masses, take up the dye which appears abundantly in certain of the lutein cells, theca cells, and young macrophages. The phagocytic nature of these senile macrophages might be doubted were it not for the fact that in frozen sections of such ovaries, and in paraffin sections of ovaries fixed in Susa's fluid, one can readily observe all transition stages between active macrophages containing much dye but little or no pigment and large cells heavily loaded with these pigment globules, many of which are surrounded by a halo of small Trypan blue granules. A gradual change in the nuclear pattern from the firm outline of normal phagocytes to a much wrinkled and collapsed outline in later stages of this transition, and frequent coalescence of these cells to form binucleate and multinucleate giant cells (Fig. 12) also indicate that these macrophages are gradually filling with pigment globules and undergoing physiological senescence.

In E deficient ovaries fixed in Zenker-formol and post-osmicated, the interstitial cells are everywhere intensely blackened; the macrophages vary from light to dark brown, due to the presence of small lipid globules (between or within the pigment inclusions) which appear to be no more abundant than in the macrophages of normal ovaries. Except for the presence of lipids and the striking tendency to fuse into binucleate and multinucleate masses, phenomena which may bear some relation to each other, these phagocytic cells of the ovary resemble in every detail those encountered in the uterus and elsewhere.

Ovaries from rats maintained in a state of chronic vitamin A deficiency for periods up to 560 days of age, representing material from studies presented in a previous report (Mason[16]), have been stained by the methods used in the present study. These organs were normal in every respect and showed none of the changes characteristic of E deficient rats.

The distribution of alkaline phosphatase, as demonstrated by the method of Gomori,[8] was followed in ovaries of E deficient rats 96 to 114 days old (6 animals), and 375 to 578 days old (10 animals), and in E controls 443 to 603 days old (6 animals). In all instances the theca cells of the follicles and the walls of the capillaries and small blood vessels contained large amounts of the phosphatase, which was absent or present only in traces in the macrophages and elsewhere.

Due to certain technical difficulties in demonstrating the acid

phosphatase (method of Gomori[9]) material for satisfactory comparison is limited to two animals, one deficient and one control rat, respectively 485 and 440 days old. In both animals acid phosphatase was abundant in granulosa cells and in lutein cells, decreasing in the latter in proportion to the extent of luteal regression. It was also moderately abundant in macrophages within young atretic follicles, and present to a lesser degree in many other macrophages.

Apparently E deficiency does not significantly alter the distribution of either alkaline or acid phosphatase in the different cell types of the rat ovary. The striking abundance of alkaline phosphatase in theca cells, and of acid phosphatase in granulosa and lutein cells, seems not to have been noted in the literature. Its possible application to the controversial question concerning the thecal origin of certain lutein cells is suggested.

Response of the ovarian changes to vitamin E therapy

Our material does not permit a critical appraisal of the effect of vitamin E therapy upon the pigment deposition in the ovary. A few isolated observations have been made which, unfortunately, do not permit comparison between ovaries removed from the same animal before and after E therapy. Two rats deficient in vitamin E until the 50th day of life and then given E therapy for 3 and 4 months, respectively, had essentially normal ovaries. Two others deficient in E for 7 and 8 months and then given a high intake of vitamin E for 3 to 4 months showed ovarian conditions more severe than would have been expected to exist at the institution of therapy (Fig. 4; cf. Figs. 3 and 5).

However, on careful examination macrophages which are, or seem to have recently been, related to atretic follicles usually show increased vacuolation of their cytoplasm and a relatively healthy appearance in general. This might be interpreted as indicating that the products of cell degeneration more recently acquired by them are more readily broken down than were those presented to their predecessors; the latter cells, having fused to form giant cells or having for other reasons lost their ability to escape by way of the lymphatics, have become stranded within the organ. The general picture is suggestive of a slow restoration of normal processes concerned with elimination of the products of cell degeneration in the ovary, associated with and confused by products resulting from a previous disturbance of these processes.

The testes of normal rats

In the testes of normal rats no pigment occurs except for small brownish globules of waste pigment, generally regarded as lipofuscin, in the Leydig cells of the interstitial tissue. The intertubular areas contain moderate numbers of macrophages which can readily be differentiated from the Leydig cells by their capacity to phagocytize Trypan blue and other vital dyes following their subcutaneous or intraperitoneal injection over a period of several days (Evans and Burr[6]). When stained by the Turnbull blue-basic fuchsin method these macrophages show a faintly pink, cloudy, rather homogeneous cytoplasm usually containing traces of iron.

The testes of vitamin E deficient rats

A gross brownish discoloration of the rat testis after prolonged E deficiency has been reported (Evans and Burr,[6] Martin and Moore[14]), and observed by one of us (K.E.M.) for many years, but no explanation has been offered for its occurrence. However, application of those technics which proved so effective in studying pigment distribution in other organs and tissues has revealed in the macrophages of the interstitial tissue a widespread accumulation of pigment globules which appear identical to those found elsewhere in the E deficient rat. These cells, which are somewhat increased in number and widely scattered in the interstitial tissue (Figs. 13 and 15), are relatively uniform in size and not much larger than are those of control rats. Larger cells occasionally occur in clusters in the vascular connective tissue just internal to the capsule (Fig. 14). Their size, staining characteristics, and tendency to form binucleate and small multinucleate cells indicate that they are senescent macrophages whose escape into the lymph sinusoids has been retarded or prevented.

In teased preparations from testes of rats injected with Trypan blue, and in paraffin sections of these testes fixed in Susa's fluid, practically every macrophage contains dye granules, widely distributed between coarser pigment globules of varied size. These physiologically active cells also contain moderate numbers of lipid globules staining with Sudan IV and Nile Blue sulphate, but are invariably devoid of demonstrable iron.

Although difficult to establish upon a quantitative basis because of extensive atrophy of the seminiferous tubules, there appears to be

at least a two-fold increase in the number of macrophages. This is more obvious when such testes are compared to those of vitamin A deficient rats (representing experimental material previously described; Mason[15]) in which the total number and staining reactions of the macrophages resemble in every way those in testes of normal control rats, except perhaps for an increased amount of demonstrable iron.

It is of significance that the accumulation of pigment globules in the macrophages does not become apparent until several months after testicular degeneration is complete—a process in which the germ cells show widespread injury and, within a few weeks, are sloughed and removed to the ducts of the epididymis where they undergo gradual dissolution. Furthermore, we find no evidence that the Leydig cells, the smooth-muscle cells of the blood vessels, or the connective tissue framework of the atrophic testes represent the source of this pigment. The possibility that the macrophages of the testes absorb this pigment from the general circulation seems quite remote.

On the other hand, the Sertoli syncytium of the atrophic seminiferous tubules everywhere contains many globules of variable size which are selectively stained by basic fuchsin with about the same intensity as are the globules within the macrophages. They seem not to be products of the Sertoli syncytium but rather the degenerate remnants of germ cells which became entangled in this syncytial tissue and underwent degeneration in situ. However, it is possible that the Sertoli nuclei, whose nucleoli represent the only other structures in the testis showing similar staining affinity, may contribute to this material. The basement membrane of these tubules seems to be everywhere intact. Yet, many macrophages can be found closely applied to this membrane, often at points where the cytoplasm of the connective tissue cells is greatly thinned out and vacuolated. It seems probable that the macrophages absorb this material from the Sertoli syncytium by transfer through or between the cells of the basement membrane.

In testes of rats given vitamin E therapy for from 3 to 5 months following a period of prolonged deficiency the majority of macrophages still contained scattered globules of pigment, but the latter showed a striking reduction over that present in animals given no E therapy. It seems logical to assume that the macrophages present before therapy began escaped via the lymphatics and were replaced

by others whose pigment content is much less. There also appears to be a very definite reduction in the amount of fuchsin staining material present within the Sertoli syncytium.

Discussion

On the basis of staining reactions, and the unique inability of tissue macrophages effectively to autolyze and degrade it, the pigment occurring in the ovaries and testes is identical to that so abundant in the uteri and other organs of long-term E deficient rats. The large size of the globules, their resistance to strong acids, strong alkalis, fat solvents and oxidizing agents, and their staining affinities, suggest an exceptionally stable protein-lipid combination. The observations presented here and elsewhere (Mason and Emmel[18]) indicate that a clear understanding of the chemical nature of this pigment would provide a valuable clue to the identity of some cellular enzyme system, or systems, in which vitamin E plays a vital rôle.

The increased accumulation of pigment in the ovary is not associated with any alteration in the histological structure or physiological functions of the organ, other than those changes incident to senility or to physical debility in some animals showing rather advanced muscular dystrophy. It has also been the experience of many other investigators that the ovary suffers no ill effects in long-term E deficient rats, as judged by regular estrus and normal mating behavior. The increased need for vitamin E to secure successful gestations as the deficiency is prolonged (Barrie,[1] Emerson and Evans,[4] Evans and Emerson[7]) seems not related to any ovarian dysfunction, but probably to the uterine changes referred to previously. As far as we can determine, the accumulation of pigment in the ovary is not influenced by the occurrence of repeated absorptions, as seems to be true of the uterus (Barrie,[1] Hessler,[10] Sweeten[21]).

Unlike the situation in smooth-muscle cells which produce and liberate the pigment material without suffering necrosis, the pigment of the ovary arises by a process which resembles that in striated muscle and cardiac muscle. In these muscles the pigment is released to the macrophage only during or after necrosis of the muscle fibers; globules being readily discernible, however, in muscle cells which are in process of necrosis (Mason and Emmel[18]). In the ovaries of our E deficient rats the atretic follicles and the corpora lutea showing

advanced regression contain many scattered cells which are obviously pyknotic and have the same intense staining reactions shown by macrophages in adjacent regions which have been, and still are, engaged in their removal.

As in ovaries of control rats, macrophages concerned with the process of follicular atresia greatly exceed those involved in the slow regression of corpora lutea. When due allowance is made for variations in the ovarian picture incident to the estrous cycle, there is nothing to indicate that either follicular atresia or luteal regression is disturbed by E deficiency. The effect of the deficiency state first becomes evident as either an excess of a normal product or as a distinctly abnormal metabolite, released by the cells undergoing degeneration in the course of these two processes. Although we find no clear-cut evidence of pigment globules in these cells prior to the onset of degeneration, it is quite possible that they experience a metabolic change which, while not materially affecting their functional or structural integrity, alters the character of their decomposition products. Only the degenerating ova contain granules resembling, in staining reactions, those seen in the macrophages. Yet the number of the latter in the ovaries seems too great to be accounted for on the basis of origin from ova alone.

Undoubtedly many of the macrophages escape from the ovary and contribute to the subsequent dispersal of this pigment to the lymph nodes, spleen, and liver. Others, however, laden with pigment globules, lose their motility and become permanently stranded within the ovary. Their tendency to fuse into giant cells, an evidence of senescence, may be related to their content of lipids—a feature in which they, and those of the testis, differ from the macrophages elsewhere in the E deficient rat.

Large brown macrophages have been observed in ovaries of mice after prolonged treatment with gonadotrophins (Pfeiffer and Hooker[19]), and in testicular tumors of mice induced by prolonged administration of estrogens (Hooker and Pfeiffer[11]). The ovarian cells were regarded as macrophages containing hemosiderin, the testicular type as macrophages concerned perhaps in the removal of hypertrophied Leydig cells; both resembled the "brown degeneration" cells characteristic of the adrenal glands. The latter type of cell is present to the same extent in the adrenals of our deficient rats as in those of the controls. Although the origins of the ovarian and testicular macrophages referred to above are apparently quite

different from those present in our deficient rats, a comparison of their staining and cytological characteristics should prove of interest.

The pigment arising in E deficiency is not lutein, which occurs in the ovary of some species but not in the rat; nor is it a melanin pigment, for the cells giving rise to it and the macrophages containing it have consistently failed to give a positive dopa-oxidase reaction. Although its staining reactions are characteristic of hemofuscin, there is no reason to suspect a hematogenous origin. In many respects it resembles the so-called "ceroid" pigment occurring in the liver of rats suffering from nutritional cirrhosis (Endicott and Lillie,[5] Popper, György, and Goldblatt[20]). It is hoped that studies now in progress will permit more satisfactory characterization of this pigment.*

Summary and conclusions

1. With progressive deficiency of vitamin E in rats there occurs a gradual accumulation of pigment in the ovaries and testes, similar to that recognized as characteristic of the uterine and other smooth muscle and the skeletal muscle.

2. The ovarian pigment (which apparently occurs in normal ovaries to a limited extent) is regarded as an abnormal metabolite or intermediate breakdown product, probably an unusually stable protein-lipid combination, liberated in an otherwise normal process of cell degeneration in atretic follicles and regressing corpora lutea. Tissue macrophages, which phagocytize but cannot assimilate these inert pigment globules, slowly accumulate and undergo senescence in the interstitial tissue; some escape through the lymphatics. Vitamin E therapy does not accelerate removal of the pigment, but it may slowly decrease the amount liberated in the necrosis of subsequent generations of cells.

3. The amounts of histologically demonstrable inorganic iron, alkaline phosphatase, and acid phosphatase are essentially the same in the ovaries of E deficient and control rats. The dopa-oxidase reaction for melanin is consistently negative.

4. Several months after the characteristic degeneration of the germinal epithelium has occurred macrophages of the testicular

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interstitial tissue increase in number and become loaded with pigment globules. This pigment appears to represent cell remnants of the germinal epithelium which have become entangled in the Sertoli syncytium and are later phagocytized by the macrophages. Prolonged vitamin E therapy greatly reduces the pigment content of the macrophages in the testis.

5. Prolonged, chronic deficiency of vitamin A causes no formation of pigment in the ovaries or testes of rats.

6. The ovarian and testicular pigment is identical to that appearing in striated and smooth-muscle cells. In mode of origin it more closely resembles that observed in the striated musculature.

7. Further characterization of this pigment, histologically and chemically, might provide an important clue to some particular cellular enzyme system, or systems, whose physiological integrity depends upon the presence of vitamin E.

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