Lipofuscin Distribution and Histological Lesions in the Vitamin E Deficient Cotton Rat (*Sigmodon hispidus hispidus*)

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Summary. To identify the role of avitaminosis E in the production of histological lesions and the distribution of an acid-fast pigment in the young cotton rat, weanling animals were maintained on a vitamin E deficient diet for 41 to 179 days. Since the pigment, lipofuscin, cannot be degraded by any cell, including the macrophage, it accumulates linearly with age of the animal and the length of the avitaminosis E.

Significant amounts of lipofuscin collect in the adrenal cortex, ovary, uterus, skeletal muscle and liver. Lesser concentrations are found in the cardiac muscle, testis, prostate, spleen, lymph nodes, pancreas, fallopian tube, kidney and bone marrow.

Traces of acid-fast pigment occur in the older normal control animals as a natural phenomenon in aging. However, in avitaminosis E this process is accelerated and intensified. With the addition of tocopherol to the deficient diet only a few acid-fast granules appear. These findings suggest that vitamin E may delay cellular aging as supported by the marked abnormal accumulation of acid-fast (old age) pigment in the tissues of vitamin E deficient animals.

Histopathological lesions are found in skeletal muscle, heart, kidney and liver of all vitamin E deficient animals.

The occurrence of yellow-brown, acid-fast pigment in various tissues of animals deficient in vitamin E has been extensively documented since its striking accumulation in the uterine smooth muscle in the vitamin E deficient rat was described by Martin and Moore, 1939. It is often referred to as “ceroid”, a term applied to a similar pigment observed in cirrhotic livers of rats fed diets low in protein and fat (Lillie, et al., 1941). The extensive literature on these pigments has been the subject of several reviews (Einarson and Telford, 1960; Trautwein, 1962; Howes et al., 1964; Norkin, 1966; Patek et al., 1967). In dietary studies on avitaminosis E in the cotton rat we observed extensive lipofuscinosis as well as considerable tissue pathology. This report documents for the first time the occurrence and distribution of acid-fast lipofuscin in the vitamin E deficient cotton rat with or without histopathological lesions.

Materials and Methods

Seven male and nine female weanling cotton rats were reared on a vitamin E deficient diet composed of casein (20%), corn starch (50%), lard (18%), brewer’s yeast (7.5%), salt mixture (2.5%), and cod liver oil (2%). The experimental controls were four animals fed the same diet and received a diet supplement of mixed natural

*Data from S. R. Swensen’s Master of Science thesis, Department of Anatomy, George Washington University.
tocopherols providing 0.25mg of alpha tocopherol daily. Four animals on normal diet served as normal controls. At sacrifice after varying periods on experiment, (41–207 days) tissues were fixed in Zenker’s solution, sectioned in paraffin at 5μ, and stained with carbolfuchsin (Kinyoun’s formula) for acid-fast pigment followed by Ehrlich’s hematoxylin.

**Results**

The distribution of lipofuscin, in the tissues and organs studied, is summarized in Table 1.

**Table 1.** Distribution of acid-fast pigment in chronic vitamin E deficient and normal cotton rats

<table>
<thead>
<tr>
<th>Organs**</th>
<th>No. of Rats</th>
<th>41–90 days</th>
<th>91–140 days</th>
<th>141–179 days</th>
<th>No. of Rats</th>
<th>Deficient diet plus tocopherol 100 days</th>
<th>207 days</th>
<th>Normal diet 175–182 days</th>
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<td>2 ±</td>
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<tr>
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<td>8</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>2</td>
<td>−</td>
<td>+</td>
<td>2 −</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cortex</td>
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<td>−</td>
<td>+</td>
<td>++</td>
<td>2</td>
<td>−</td>
<td>+</td>
<td>2 −</td>
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<tr>
<td>medulla</td>
<td>5</td>
<td>± to +</td>
<td>+ to ++</td>
<td>++ to +++</td>
<td>2</td>
<td>−</td>
<td>±</td>
<td>2 −</td>
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<td>Adrenal</td>
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<td></td>
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<td>z. glom.</td>
<td>9</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>4 −</td>
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<tr>
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<td>+ to ++</td>
<td>± to ++</td>
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<td>−</td>
<td>−</td>
<td>4 −</td>
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<tr>
<td>z. retic.</td>
<td>9</td>
<td>+ to +++</td>
<td>+ to +++</td>
<td></td>
<td>4</td>
<td>+</td>
<td>±</td>
<td>4 − to ±</td>
</tr>
<tr>
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<td>9</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>4 −</td>
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<td>14</td>
<td>±</td>
<td>+</td>
<td>++</td>
<td>4</td>
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<td>±</td>
<td>4 −</td>
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<td>−</td>
<td>−</td>
<td>4 −</td>
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<td>±</td>
<td>± to +</td>
<td></td>
<td>4</td>
<td>−</td>
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<td>4 −</td>
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<td>±</td>
<td>±</td>
<td>2 −</td>
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<td>Prostate</td>
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<td>±</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>2 −</td>
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<td>±</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>−</td>
<td>±</td>
<td>4 − to ±</td>
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<td>Lymph nodes</td>
<td>12</td>
<td>±</td>
<td>± to +</td>
<td></td>
<td>4</td>
<td>−</td>
<td>±</td>
<td>4 − to ±</td>
</tr>
<tr>
<td>Pancreas</td>
<td>13</td>
<td>±</td>
<td>+</td>
<td></td>
<td>4</td>
<td>−</td>
<td>±</td>
<td>4 − to ±</td>
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<td>Fallopian tube</td>
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<td>±</td>
<td>±</td>
<td>+</td>
<td>2</td>
<td>−</td>
<td>±</td>
<td>2 ±</td>
</tr>
<tr>
<td>Kidney</td>
<td>11</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>4 −</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>14</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>4</td>
<td>±</td>
<td>±</td>
<td>4 −</td>
</tr>
</tbody>
</table>

* Pigment score — None, ± Trace, + Slight, ++ Moderate, +++ Considerable, +++ Extensive.
** The following organs were free of acid-fast pigment; lung, esophagus, stomach, intestines, salivary glands, thyroid, trachea, penis, vagina, epididymis, seminal vesicle, urinary bladder and preputial gland.
Fig. 1. After 70 days of avitaminosis E uterus has only a few pigment granules in stroma.

Fig. 2. After 96 days increased amounts of pigment in stroma and epithelium of uterus.

Fig. 3. After 129 days marked increase and size of pigment globules in uterus. All tissues stained with carbol-fuchsin and Ehrlich’s hematoxylin. ×605
The endometrial stroma and uterine epithelium contained marked acidophilic pigmentation, increasing in direct proportion to the length of time on the vitamin E deficient diet. After 50 days, large coarse acid-fast globules (+ and ++) appeared in the endometrial epithelium. In the stroma there was a similar distribution but the size and number of the globules were decreased. For the most part the pigment appeared within macrophages in distinct, homogeneous patches, but it was also found within the stromal cells. Occasionally extracellular pigment was observed.

In animals maintained for 75 days on a vitamin E-deficient diet, there was an intracellular increase of lipofuscin accumulation in the endometrial stroma (Fig. 1). In the 96 day deficient rat, the pigment in the epithelium and stroma increased further in concentration (++ to +++)(Fig. 2). Extracellular pigment as well as pigment-filled histiocytes were abundant. The clumped pigment stained a smudgy, brick-red with irregular areas of intensified brilliance.

The uteri of the 129, 144 and 154 day deficient animals demonstrated further increased areas of intense staining. The uteri of these animals demonstrated further increased coalescence of pigment globules within giant macrophages (Fig. 3). In the myometrium only occasional pigmented macrophages were observed. The smooth muscle fibers were histologically normal, in marked contrast to our earlier findings in other animals (Mason and Telford, 1947; Einarson and Telford, 1960).

The uteri of the 100 day vitamin E supplemented animals revealed little, if any, lipofuscin. The uterus of a 207 day supplemented rat had a + reading in both the epithelium and stroma. The normal control animals displayed only a trace of pigment in the endometrial stromal cells.
Ovary

In the 50 day deficient animals, traces of pigment were seen within the medulla. The acid-fast material was located in the cytoplasm of macrophages as well as small, extracellular globules. In animals longer on the diet (96 days), the amount of pigment generally increased (+ to ++++) (Fig. 4) and was no longer confined to the medullary regions but extended into the cortical areas. Lipofuscin carrying macrophages increased in number and assumed large irregular shapes. The extracellular globules increased also in number and size. Occasional pigmented areas were seen within the corpora lutea but the developing follicles were not pigmented. With the exception of the follicles, ovaries of 129 and 154 day deficient rats were largely filled with intracellular pigment (+++ to ++++).

The rats receiving the deficient diet plus 0.25 mg/day tocopherol for 100 and 207 days, were not entirely free of pigment in the ovary. Limited amounts (+) were found within the cells of the ovarian medulla. The normal control animals had little or no acid-fast material within the ovarian medulla or cortex.

Adrenal

In the animals held on the vitamin E deficient diet for 50 to 179 days, there was
an increase of lipofuscinosis in the adrenal cortex directly proportional to the length of the avitaminosis.

In the 50 day deficient rats, small granules of red stained pigment (+) appeared within a few of the parenchymal cells of the zona reticularis (Fig. 5). For the most part, the pigment was intracellular, but there were some extracellular granules. There was an occasional trace of lipofuscin in the zona fasciculata. No pigment was seen in the zona glomerulosa or in the medulla.

Later in the deficiency (90 days), a most striking increase of pigment (++) occurred in the zona reticularis (Fig. 6). Acidophilic masses appeared within the parenchymal cells in different sizes and shapes, varying from minute granules to large, homogeneous globules, causing the reticularis cells to enlarge several times their normal size. Extracellular pigment, having the same texture as the intracellular pigment, was seen infrequently. In the later stages of the deficiency (140 to 179 days) almost every cell in the zona reticularis was filled (+++) with acid-fast masses (Fig. 7).

In the early stages of deficiency a rare trace of pigment could be seen in the cells of the zona fasciculata. This increased to + in the 96 day and to ++ in the 129 day deficient animals (Fig. 6). Most of the lipofuscin was intracellular but caused no cellular enlargement, as seen in the zona reticularis.

A trace of pigment was first seen in the cells of the zona glomerulosa in the 96 day deficient cotton rat. Although all of the latter stages of avitaminosis E demonstrated lipofuscin accumulation it never progressed beyond the trace level. By the 146th day of the deficiency pigment granules continued to concentrate in the zona reticularis (++) and the zona fasciculata (++) but still only a trace was found in the zona glomerulosa (Fig. 7). Most of the lipofuscin was intracellular causing cells, especially in the zona reticularis, to become greatly distended.

The medulla remained completely free of pigment in all stages of deficiency. Only a trace was seen in the 207 day tocopherol supplemented animals. All cortical zones were completely free of lipofuscin in the normal rats except one female, which had a trace in the cells of the zona reticularis.

Liver

The livers of all deficient animals were pathological to some degree. Cloudy swelling of the hepatic parenchymal cells, ranged from slight to severe. In 14 of the 16 vitamin E deficient animals, there was also slight to extensive fatty infiltration. In the earlier stages of deficiency the hepatocytes were swollen with many small fat vacuoles in the cytoplasm. In the older deficient animals, this condition progressed to mature signet-ring fat cells and complete derangement of hepatic cords around the central veins. In the oldest deficient animals, the fat cells appeared to coalesce and the hepatic cells degenerated into granular masses without cellular outline.

In the younger deficient animals a trace of acid-fast material was seen in the hepatocytes around the hepatic triad. In the later stages of vitamin E deficiency, the amount of pigment increased to a + and ++, and was still seen mostly around the hepatic triads.

One of the 100 day supplemented animals had extensive fatty infiltration and slight pigmentation of hepatocytes, while the other 100 day treated rat had no abnormal apparent fat or pigment accumulation. The 207 day supplemented animals
had some fatty change and a trace of lipofuscin. There was no fatty metamorphosis or pigment in the liver cells of either the young or the old control rats.

**Skeletal muscle**

Sections were taken of the gastrocnemius, thigh adductors, pectoralis major, psoas major, diaphragm and intercostal muscles.

The earliest microscopic lesion of tocopheral deficiency in the cotton rat was recognized in skeletal muscles. All of the vitamin E deficient animals revealed extensive degenerative lesions, increasing in severity in those rats longer on the deficiency.

The first appearance of significant amount of lipofuscin occurred after 75 days of the vitamin E deficiency. It was observed within the muscle fiber and in macrophages associated with the endomysium and the perimysium. The largest amount of pigment accumulated after 130 days of deficiency and remained quite constant thereafter.

Muscle fibers were frequently seen without cross striations but had typical Zenker-type degeneration with many swollen and hyaline fibers (Fig. 9); other fibers were shrunken and fibrotic (Fig. 8). The rounded muscle nuclei with multiple nucleoli were increased in number and lined-up in rows within the sarcolemma (Fig. 10). Regenerating fibers with centrally placed nuclei were observed (Fig. 8). In areas of

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**Fig. 8.** Pectoralis major muscle of 50 day E-deficient animal. Fibers 1, 3 and 5 shows complete degeneration. Fiber 2 is a newly regenerated fiber. Fiber 4 is shrunken and atrophic. Fiber 6 is a normal, mature fiber. Only a few scattered pigment granules present. \(\times 275\)

**Fig. 9.** Adductor magnus muscle after 50 days of deficiency. Upper fiber undergoing Zenker's or hyaline degeneration. Lower fiber shows loss of cross striations and central rowing of nuclei. Some pigment granules present. Carbol-fuchsin and Ehrlich's hematoxylin. \(\times 400\)
necrosis there was lymphocytic infiltration (Fig. 11). Small clear vacuoles, fat droplets and acid-fast lipofuscin were seen within the degenerating sarcoplasm and in macrophages (Fig. 11).

In the vitamin E supplemented animals, skeletal muscle was comparatively normal with only a few damaged fibers and mild infiltration of lymphocytes. No acid-fast granules were found. No pigment or damaged fibers were observed in the striated muscles of the normal control cotton rats.

Cardiac muscle

Similar pathologic changes seen in skeletal muscle were also observed in cardiac muscle, but they appeared later in vitamin E deficiency and were usually not as pronounced. However, pyknotic nuclei and vacuolization of the sarcoplasm were more marked than in skeletal muscle. In the more prolonged deficiency, the fibrotic replacement of myofibrils was very evident. In the deficient animals from day 96 to
Lipofuscin in Vitamin E Deficient Cotton Rat

179, seven of the nine rats had a trace to a slight amount of pigment located in macrophages and in small globules around areas of necrosis.

No heart lesions or pigment accumulation were found in the supplemented or normal control animals.

**Testis**

Minute, round globules of acidophilic lipofuscin appeared in the seminiferous tubules of the vitamin E deficient rat. In the younger animals the acid-fast droplets of varying size were situated, for the most part, just inside the basement membrane among the spermatogonia. In the later stages of the deficiency, the globules increased in number (+) and were diffusely scattered extracellularly throughout the tubules. A small amount of acid-fast material was also present in the interstitial cells of all deficient rats. Unlike that found in the seminiferous tubules, the pigment was intracellular and had a granular, somewhat smudged appearance.

Testes from the supplemented animals displayed traces of pigment. None was found in the normal cotton rat testis.

**Lymph nodes**

In the lymph nodes of almost every deficient animal, small traces of pigment could be observed. The material was not equally dispersed throughout the nodes, but was located in discrete foci, mostly within the medullary sinusoids. Most of the lipofuscin was located in macrophages, although some distinct extracellular globules of pigment were observed. A direct proportional increase of acid-fast granules with longer maintenance on the vitamin E deficient diet was evident.

Most of the lymph nodes of the supplemented group displayed traces of pigment in macrophages within the medullary sinusoids. The normal control animals had only a trace of acid-fast pigment.

**Spleen**

By the 75th day on the deficient diet animals showed traces of lipofuscin in the spleen. Unlike the discrete foci of pigmentation of the lymph nodes, the material was more diffusely scattered. It appeared as small globules within numerous macrophages and also as occasional small, distinct extracellular masses.

Most of the pigment was located in macrophages of the red pulp. The amount increased from a trace to a slight amount in those animals who were fed the vitamin deficient diet for 120 to 179 days.

Three of the four supplemented rats showed no acid-fast substance within the spleen. The fourth displayed only a trace. One of the young controls had a trace of lipofuscin. The other controls were completely negative.

**Pancreas**

In the vitamin E deficient animals round, globular accumulation of acid-fast bodies were seen largely within the pancreatic acinar cells. The globules within the acini were made-up of an acidophilic, homogeneous material—found mostly in the pyramidal epithelium and occasionally in the centroacinar cells. Infrequently similar globules were seen extracellularly in the interstitial space. An occasional
pigment-laden macrophage was also observed. Trace amounts of lipofuscin were seen in the earlier stages of deficiency, but by the 129th day, most acinar cells had a slight accumulation of pigment. The islets of Langerhans were pigment-free in all sections.

One supplemented and one normal control rat showed a trace of pigment in the acini and interstitial space. The other animals of these control groups were free of pigment.

**Kidney**

All deficient animals had normal appearing glomeruli but in each animal the proximal, distal and collecting tubules demonstrated tubular necrosis with the epithelium undergoing cloudy swelling and hydropic degeneration.

Kidneys of four animals in the later stages of vitamin E deficiency (129 to 179 days), had a trace of lipofuscin within the epithelium of the proximal and distal convoluted tubules. The other deficient animals, as well as the vitamin E supplemented and control groups, were free of such pigment.

**Bone marrow**

In the red bone marrow of the sternum pigment was first found in a 96 day deficient animal. In nine rats held on 96 to 179 days of avitaminosis E, only six had traces of pigment within macrophages.

Two of the four animals in the supplemented group had some pigmented macrophages in the bone marrow similar to those in the deficient group. Normal control rats were completely free of pigment.

**Other organs**

In the vitamin E deficient animals the salivary glands, thyroid, thymus, trachea, lung, gastro-intestinal tract, penis, vagina, seminal vesicle, epididymus, bladder and preputial gland had normal appearing tissues with no demonstrable lipofuscin. In the vitamin E supplemented and control animals these organs were also morphologically normal with no pigment present.

**Discussion**

Our study of metabolic lesions of vitamin E deficiency in the cotton rat, agrees with the earlier observations of Martin and Moore (1939), Mason and Emmel (1944, 1945) and Mason and Telford (1947) that laboratory rats demonstrate varying degrees of lipofuscinosis, principally in the uterus, ovaries, testes, adrenal cortex, liver, skeletal and cardiac muscle. The acid-fast material appears and stains much like the wear-and-tear pigments which are observed in the zona reticularis of the adrenal cortex, uterus, ovary and elsewhere in the body of the presumably normal animals (Mason and Emmel, 1945). This pigmentation is believed to be a natural process of aging and not necessarily related to diet.

The source of the pigment, lipofuscin, is probably from the intracellular peroxidation of phospholipids and unsaturated fatty acids. The exact genesis of the pigment granules is obscure. However, it has been suggested that they are, (1) the remnants of degenerated mitochondria or lysosomes, (2) unique organelles which
sequester harmful intracellular garbage, or (3) products formed by cross-linking of elements of the endoplasmic reticulum (Kohn, 1971). In any event the pigment is produced and stored within the cell. Yet we observed in several organs variable amounts of extracellular lipofuscin. Such a finding may be explained by the recent lysis of a cell (e.g. muscle fiber) with the release of pigment granules in the immediate area. Also perhaps the rupture of the plasma membrane of a pigment engorged macrophage would cause a spewing out of acid-fast debris which would later be rephagocytized by other histiocytes. Since the amount of extracellular pigment is minimal in most cases, the time interval between release of the pigment and its subsequent phagocytosis, is probably quite brief.

In studying the tissues of chronic vitamin E deficient cotton rat, one is impressed with the progressive development of the acid-fast material. The primary development and appearance of lipofuscin is located in the epithelial and stromal cells of the uterus and ovary, in the parenchymal cells of the adrenal cortex and liver, and in skeletal muscle fibers. Small granules accumulate intracellularly and increase in size with time. The granules coalesce into globules and reach such proportions that the cell may become swollen with pigmented masses, especially in the adrenal cortex. Because of the pigment or some other factor, the distended cells undergo autolysis and the pigment spills into the interstitial areas where macrophages phagocytize it. It appears that the macrophages are unable to autolyze the ingested material and they increase in size. Eventually they die and their contents are ingested by other macrophages, which likewise suffer a similar fate. In this manner large globules of pigment accumulate in discrete areas. The longer the period of vitamin E deficiency, the greater the amount of pigment is seen.

Macrophages carrying acid-fast lipofuscin are seen in lymph nodes, spleen, bone marrow and pancreas. Otherwise these organs appear to be histologically normal and not necessarily involved in pigment production. Possibly the pigment in these areas has been brought in by phagocytes from areas of primary development elsewhere.

Our study is in basic agreement with other investigations that animals fed normal diets or having received vitamin E deficient diets plus a minimal supplement of alpha tocopherol demonstrated little or no accumulation of acid-fast staining pigment within organs (Mason and Emmel, 1944, 1945). They have shown that uterine pigmentation can be prevented with tocopherol administration and that pigmentation due to avitaminosis E can be arrested with supplementary vitamin E.

In the cotton rat specific organs are actively accumulating lipofuscin in the early stages of vitamin E deficiency. Depending upon presence or absence of cellular necrosis they can be divided into two groups.

Group 1. Although massive amounts of pigment are present in the ovary, uterus, testis, and adrenal cortex, there is no apparent cellular necrosis.

Group 2. In the liver, skeletal and cardiac muscle, the pigment concentration is accompanied by marked cellular damage.

In the first group, a striking accumulation of pigment occurs. The common denominator of these organs is that they are all endocrine glands or target organs of the anterior pituitary. It has long been known that vitamin E deficient animals have difficulty in normal reproduction. Although the female rat is able to conceive,
extensive intra-uterine fetal death and resorption occur (Evans and Bishop, 1922). The male rat is sterile (Evans, 1925) because of abnormal spermatogenesis, nonmotile sperm, and seminiferous tubular degeneration. Grossly there is atrophy of the testis (Howes, et al., 1964). In other studies acid-fast pigmentation was not observed in Leydig cells until testicular degeneration was complete. Most of the pigment was observed in macrophages (Mason and Emmel, 1944). None of these phenomena is seen in the cotton rat testis. In this study all stages of normal spermatogenesis are observed in the experimental animals. Also small extracellular pigment globules are seen in the seminiferous tubules and among the interstitial cells. Pigmented macrophages are rare.

The uterus of the vitamin E deficient cotton rat has a very heavy infiltration of pigment in the endometrium. The question must be asked, if this is an expression of some disturbed metabolic function. It is known that vitamin E deficient rats resorb their fetuses. However, deficient rats experiencing two or more litter resorptions, differ little in degree of uterine pigmentation from that seen in virgin E-deficient rats of comparable age (Mason and Emmel, 1945). Similar metabolic lesions occur in virgin cotton rats.

The anterior pituitary has been implicated as cause of the above pathologic conditions. Barrie (1937) found that in the anterior pituitary of vitamin E deficient rats, the acidophils and basophils were small and degenerative. In 1955 Beckmann found that the pituitary has the highest level of vitamin E of all organs. Further studies have shown that given tocopherol, sterile animals were able to reproduce (Lee, 1960) and ACTH was activated (Ichihara, 1967). Consequently, it has been suggested that vitamin E acts primarily on the function of the anterior pituitary (Ichihara, 1967). Possibly this could be the reason why certain endocrine organs accumulate pigment in vitamin E deficiency (Mason, 1944).

It has been expressed, however, that the gonado-hypophyseal dysfunction, which exists in vitamin E deficient rats, is more a result of, rather than the cause of, characteristic reproductive disturbances in the deficiency (Mason, 1944). Mason and Emmel (1944) have observed pigment accumulation in the rat without any alteration in the physiologic functions of the ovary, other than those due to senility. These pigment deposits have been seen in long term vitamin E deficient rats who displayed regular estrus and normal mating behavior, implying that changes in the pituitary and adrenal are secondary phenomena rather than primary. Drummond et al. (1939) indicated that tocopherol has no gonadotropic effects. They administered vitamin E to immature female rats and hypophysectomized adult females and observed no estrogenic effect on ovaries or uterus. They also demonstrated that vitamin E deficiency symptoms could not be prevented with administration of gonadotropic hormones. Thus they proved that the lesion is not in the pituitary gland. It appears that the exact mechanism of selective endocrine organ pigmentation remains obscure.

A defect in cholesterol metabolism is a constant finding in muscles of vitamin E deficient monkeys (Morris et al., 1966). In addition, corticosterone synthesis in adrenal glands of vitamin E deficient rats is markedly inhibited (Kitabchi, 1964). Such an imbalance of cholesterol could conceivably influence the steroid hormones of the adrenal cortex, ovary, and testis. Our study has shown that adequate vitamin E in the diet will prevent a rapid increase of pigment and avoid degeneration and
autolysis of adrenal cells in cotton rats. Similar findings were reported by Tobin and Birnbaum, (1947) for Swiss albino mice.

The second group of tissues that stores pigment consists of skeletal and cardiac muscle and liver. It will be recalled that the major difference from the first group is that pigmentation occurs in the second group only with frank tissue necrosis. Because of the large muscle mass in the body, most of the fuchsin-staining pigment accumulates here.

Skeletal muscle is perhaps the most susceptible tissue in the body to vitamin E deficiency. It is here that the earliest histopathologic change is seen. The cotton rat myopathy appeared similar to that described by numerous researchers for other animals, characterized with extensive degenerative lesions, progressively increasing in severity in those animals longest on the deficient diet. However, appearance of pigment in the cotton rat did not occur at the initial onset of fiber degeneration but was seen later in the deficiency. The pigment granules were observed first within the sarcoplasm of degenerating muscle fibers. In the areas of necrosis, macrophages invaded the area and phagocytized the cellular debris as well as the acid-fast material. In the three to five month deficient cotton rat, the amount of pigment seen was considerably less than the extensive accumulation observed in the uterus, ovary and adrenal cortex.

We have no explanation for the appearance of tubular necrosis in the kidneys of cotton rats used in this study. Evidence has been presented to indicate that renal lesions, previously attributed to tocopherol lack, were actually the result of post-mortem autolysis which is more rapid in tocopherol deficient animals than in vitamin E supplemented animals (Stowe and White, 1963). Rapid fixation of our tissues prevented such autolysis.

In other vitamin E deficient mammals acid-fast substances have been described in smooth muscle of the uterus, fallopian tube, vagina, prostate, vas deferens, ureter, small intestine (Mason and Emmel, 1945), arteries and veins (Mason and Telford, 1947). Albino rats who have been on vitamin E deficient diets for 575 days, have remarkably normal uterine muscle fibers, except for large accumulations of pigment. Somehow the smooth muscle cell is able to release the pigment causing little or no necrosis (Mason and Emmel, 1944). Except for traces of pigment in the myometrium there is essentially no pigmentation of any smooth muscle mass in the E-deficient cotton rat. It is entirely possible that a 3-4 month period of vitamin E deficiency in the cotton rat is not sufficient time to cause pigment accumulation in smooth muscle.

The exact mechanism of pigment formation and tissue degeneration in vitamin E deficiency is unknown. Many investigators believed that the pigmentation results from the dysfunction of vitamin E as an antioxidant. The biological function of vitamin E as a lipid antioxidant has been a topic of research since the discovery of its antioxidant properties by Olcott and Mattill (1941).

Antioxidants prevent the oxidation of unsaturated glycerides until they themselves have been destroyed. Cellular membranes contain very labile, oxygen-susceptible, polyunsaturated lipids. Vitamin E is most important in maintaining their integrity (Olson, 1967).

When radiant energy passes through an animal cell and strikes a polyunsaturated lipid, two things can happen. If enough vitamin E is present, the radiation will
have little effect. However, when electrons collide with a lipid molecule without tocopherol protection, a hydrogen atom can be detached. This creates an unstable molecule and initiates the peroxidation of polyunsaturated lipids (TAPPEL, 1962, 1967). Peroxidation by heat, light and chemical interactions involves the direct reaction of oxygen and lipid to form free radical intermediates. The free radical is propelled at random by a strong force until it strikes another molecule. This collision creates another radical and can cause damage to cytoplasmic structures or cellular membranes, causing malfunction of a normal physiologic mechanism (TAPPEL, 1967).

An example of relatively simple membrane breakage is the increased hemolysis of erythrocytes upon exposure to H$_2$O$_2$ in vitamin E deficient animals. The theory is that vitamin E is capable of preventing the oxidation of the RBC membrane lipids when the cell is exposed to hydrogen peroxide. Absence of the vitamin leads to membrane lipid destruction and subsequent hemolysis (BINDER et al., 1965). However it should be recalled that radical formation is a natural occurring event in normal tissues and is considered a phenomenon of aging (PARKHURST et al., 1964). Perhaps then vitamin E may be considered to be an anti-aging agent.

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**References**


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