SOME EFFECTS OF PROLONGED VITAMIN E DEFICIENCY IN THE RAT

By ARCHER JOHN PORTER MARTIN, Ph.D. and THOMAS MOORE, D.Sc., Ph.D.

From the Nutritional Laboratory, University of Cambridge, and Medical Research Council

(With Plate VIII)

In an early preliminary communication we (Martin & Moore, 1936) reported that in rats maintained for prolonged periods on diets deficient in vitamin E a brown discoloration of the uterus occurred. Degeneration of the convoluted tubules of the kidneys was also found. In a more recent paper we (Martin & Moore, 1938) described a less intense discoloration in the skeletal muscles, which was localized in distribution. The muscles of the hindlegs, which displayed the paresis described by Ringsted (1935), were always noticeably discoloured. In both the uterine and leg muscles discoloration was found to be associated with muscular degeneration. The similarity of the condition in the leg muscles to the nutritional muscular dystrophy first reported by Goettsch & Pappenheimer (1931) in guinea-pigs was noted. The purpose of this communication is to give a detailed account of this work, including its most recent developments (Moore, 1939).

Experiments

Diet. Albino or piebald rats, about 1 month old, were given a diet of the following composition: Light white casein 25%, cane sugar 50%, lard 10%, salt mixture 5%, dried yeast 10%, halibut liver oil (60,000 I.U. of vitamin A per g.) 1 drop per rat per week. In our experience this diet invariably causes resorption in the first pregnancy in rats aged 3–4 months.

During most of the work control animals received the same diet with the addition of 2 or 3 drops weekly of a crude concentrate of vitamin E prepared from wheat germ oil by Glaxo Laboratories, Ltd. In more recent experiments synthetic *dl*-α-tocopherol, kindly presented to us by Messrs Roche Products, Ltd., was added.

In numerous instances determinations of vitamin A reserves were made at autopsy and adequate reserves were invariably found.

Discoloration of the uterus. Virgin female rats were killed after varying periods of restriction to the diet deficient in vitamin E. In animals killed after 3 months, about the time at which the first resorption of the foetuses occurred in mated animals, a very faint yellowish discoloration of the uterus was observed. In those killed after longer periods of deprivation, the discoloration was more marked. After one year the discoloration was invariably striking, the uterus

being a bright yellow brown or chestnut colour. In Pl. I, fig. 1, the uterus of a rat restricted to the deficient diet for about one year is compared with that of a control rat which had received the same diet with the addition of the vitamin E concentrate. Discoloration was also prevented by weekly doses of 1 mg. of *dl*-α-tocopherol, but not by 0·3 mg. doses.

After prolonged deprivation of the vitamin the oestrous cycle became abnormal, and it was impossible to render the animals pregnant.

The same discoloration was observed in the uteri of animals deficient in vitamin E which had been pregnant, and in these the organ was often enlarged and misshapened (Pl. I, fig. 2).

We have observed discoloration of the uterus with regularity in about 200 animals deficient in vitamin E, virgin and mated. No instance of discoloration has been observed in fifteen virgin animals given adequate doses of vitamin E concentrate or tocopherol, nor in a large number of animals which had reared several litters on an adequate mixed diet.

Resistance of uterine discoloration to curative treatment. In rats restricted to the vitamin E-deficient diet for periods calculated to cause marked discoloration of the uterus, and then given liberal amounts of wheat germ or wheat germ oil concentrate for periods up to 11 months, the uteri at autopsy showed no evidence of restoration to the normal colour. In one rat which was restricted to the deficient diet for 22 months, and then given 1 mg. of tocopherol daily for 7 months, the uterus was brown. In another animal which was given the deficient diet for about 8 months, followed by an adequate mixed diet for 14 months, the uterus was almost normal in colour, suggesting that some improvement may have occurred.

In animals given doses of vitamin E concentrate at an early stage in the development of the lesion normal litters were obtained, although the colour of the uterus remained abnormal after parturition.

Secondary lesions. In many animals which had been made pregnant in vitamin E determinations and subsequently restricted for further prolonged periods to the diet deficient in vitamin E, metritis, salpingitis and ovarian cysts were observed. Unlike the discoloration of the uterus, these conditions were not observed in the virgin and did not occur in all experiments. Probably they should be regarded as after effects of pregnancy combined with vitamin E deficiency.

The uterus in deficiency of vitamins other than E. In our experience discoloration is not found in rats suffering from deficiency in other vitamins. The possibility of vitamin K being involved has been ruled out by a test of our wheat germ oil concentrate. The level of dosage which prevented discoloration of the uterus in rats would have been quite ineffective in preventing K-avitaminosis in chicks of the same weight. We are deeply indebted to Dr H. Dam, of Copenhagen, for his kindness in testing our material.

Discoloration in other organs. In early stages of the deficiency, discoloration was confined to the uterus, the animal appearing otherwise in good condition.

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Later, discoloration was seen in the oviducts, and sometimes in the ovarian ligaments, possibly from altered blood pigments in the lymphatics. In advanced cases the lymph glands along the vena cava were sometimes a pale brown. The walls of the stomach and intestines and the bladder were not discoloured.

In male rats discoloration was seen in the genital organs, being most evident in the seminal vesicles. The testicles, as noted by Evans & Burr (1927), were atrophied to about half their usual length, and were of a pale yellowish brown colour on section. The seminal vesicles were also small and light tan in colour. The colloid, when present, was normal in colour, the discoloration being confined to the wall of the organ.

The discoloration of skeletal muscle is described later.

The situation of the discoloration in the uterus and seminal vesicle. When thick sections of the uteri which had been fixed in formalin were examined it was seen that the discoloration was confined to the muscular layers. On examining thinner sections and teased preparations it was found to be due to small yellow granules in the muscle cells, many of which were also in various stages of degeneration. Misshapen cells crowded with granules and three to four times the normal breadth were interspersed with cells of normal appearances. Their nuclei were swollen and distorted and stained poorly, if at all. Other cells, though retaining more normal appearances and dimensions, contained yellow granules concentrated in the middle around the nucleus. Every stage between cells with well-stained rod-shaped nuclei, surrounded by a few yellow granules, and remnants of cells represented by sausage-shaped bags full of granules occurred side by side.

The granules were most apparent when the specimen was examined in water and less obvious in media of higher refractive indices. The larger granules were rarely more than 1 μ in diameter and many of them were on the margin of resolution with an oil immersion lens. In hardened and cleared specimens mounted in balsam they were less readily seen and the colour was diminished. The granules did not stain by potassium ferrocyanide or ammonium sulphide. They were resistant to strong acids and alkali. In teased preparations dissociated with 30% potash and 10% HNO_3 they were still apparent. The appearance was reminiscent of that of cardiac muscle fibres in "brown atrophy".

The occurrence in involuntary muscle cells of yellow granules which did not turn blue with ferrocyanides was described by von Recklinghausen (1889) and later by Lubarsch (1894), Hintze (1895) and Opie (1899) in the stomach and intestines of patients dying from haemochromatosis. Von Recklinghausen invented the name haemofuscin for this pigment to distinguish it from haemosiderin, which gives the reaction for iron. He supposed it to originate from the breaking down of haemoglobin, which is characteristic of this disease, and that it was absorbed by the muscle cells and deposited in them. Opie, on the other hand, thought that the change in the involuntary muscle cells in haemochromatosis was a degeneration analogous to "brown atrophy" of the heart muscle.

The occurrence of yellow granules in involuntary muscle is not confined to haemochromatosis. Goebel (1894) pointed out that rusty patches in the involuntary muscles of the intestine were not uncommonly seen in autopsies of persons beyond middle life and became increasingly frequent with age. He showed that the discoloration was due to the deposition of yellow

granules in the muscle cells. His description of the microscopical appearances and the properties of the granules would serve equally well for those found in our rats after prolonged deprivation of vitamin E. Although he describes degenerated fibres three times the usual size, without nuclei and full of granules, and was struck with the resemblance to "brown atrophy" of the heart, he adopted, on the basis of some experimental work by Lubarsch (1894), Recklinghausen's view as to the origin of the granules. Recently, Labbé, Bonlin & Pétresco (1935) have described deposits of haemofuscin in the muscles of the intestine in bronzed diabetes.

The yellow colour in the seminal vesicles of rats long deprived of vitamin E is also confined to the muscular layers, which show changes similar to those described in the uterine muscle. Otherwise the structure of these organs seemed normal.

Nervous symptoms. Paralysis of the hindlegs, as described by Ringsted (1935), and illustrated by Einarson & Ringsted (1938), was usually well developed in animals whose mothers had received a mixed diet after restriction to the deficient diet for about 13 months. In animals born from vitamin E-deficient mothers, rendered fertile by a single dose of vitamin E, and reared on a diet deficient in vitamin E, paralysis commenced at different times, varying from a few days to several months. The animals also became emaciated and showed a tendency to skin sores. Contrary to the finding of Einarson & Ringsted all died prematurely if not killed for pathological examination. Animals which received vitamin E concentrate remained in good condition (Pl. I, fig. 3).

Observations on skeletal muscles. In rats which had shown paralysis a brown discoloration of skeletal muscles was found at autopsy. This discoloration was usually confined to the abdominal muscles and to some of the muscles of the hindlegs and lumbar region. The condition was similar to that found in the uterus but the degree of discoloration was less.

Teased preparations and sections of the affected muscles showed that many of the muscle fibres were in process of destruction. Some had lost their transverse striations and became granular. In others the nuclei under the sarcolemma were increased and the muscle substance was split longitudinally into fibrils or segments. In others the fibre was replaced by a tube composed of the sarcolemma sheath filled with proliferating sarcolemma nuclei and cells with deeply staining nuclei. The distribution of the damaged fibres was irregular, fibres in process of dissolution being intermixed with apparently normal fibres. In advanced cases of this muscular dystrophy more than 90% of the fibres of the adductor muscles of the thigh were affected. The appearances have been well described by Goettsch & Pappenheimer (1931), who published some excellent photographs, and also by Einarson & Ringsted (1938) in their recent monograph. According to the latter lesions of the spinal cord accompany the condition and they incline to the belief that the muscular dystrophy is secondary to the nerve cell degeneration in the cord. Goettsch & Pappenheimer (1931), however, stated that they found no significant alterations in the central nervous system or peripheral nerve trunks in association with muscular dystrophy in rabbits and guinea-pigs, and in a later paper Rogers, Pappen-

heimer & Goettsch (1931) come to the conclusion that the nerve endings of the degenerated muscles were preserved.

The attempted cure of paresis. We have found that rats suffering from advanced avitaminosis may sometimes survive and grow when given a diet rich in vitamin E. Eight such rats when given a diet containing 60% of wheat germ, instead of sugar, all gained in weight. In three the improvement was only temporary, death occurring after 26, 47 and 95 days. Three others died after 10 months on the new diet, but since the animals were then two years old the complication of senescence cannot be ruled out. The two surviving rats, although much heavier than before wheat germ was given, were still very undersized (218 and 220 g.) when killed after treatment for nearly 12 months. Two other rats were kept on the basal diet, but with the addition of wheat germ oil concentrate. One of them gained in weight and survived for 8 months, the other failed to respond and died in 50 days. One rat was given 1 mg. of tocopherol daily. It gained slightly in weight, but died after the treatment had been continued for 7 months without ever showing any real improvement in its condition.

Even in those rats which improved in weight and general condition paresis was never completely cured. One of the rats, which had completely lost the use of its hindlegs while receiving the deficient diet, was given wheat germ and survived in otherwise good health until the experiment was terminated but did not recover the use of its legs. In others greatly increased strength enabled them partially to overcome their disabilities. Standing and slow walking in an arched position was sometimes accomplished by using the hindlegs stiffly. At autopsy the affected muscles were still discoloured.

Kidney degeneration. Another effect of prolonged restriction to the diet deficient in vitamin E was a slow progressive parenchymatous degeneration of the kidneys, unaccompanied by any inflammatory reaction in the interstitial tissues or in the glomeruli.

We first observed this degeneration in an animal which had been 9 months on the vitamin E-free diet. It was in a moribund condition and killed by coal gas. Clear fluid was found in the pleural cavity. The kidneys were large and pale, and on microscopic examination were found to be degenerated. In subsequent autopsies the kidneys were examined as a routine. The changes in the naked-eye appearances were neither striking nor consistent, the organs being sometimes larger and sometimes smaller than normal. In a few cases concretions at the apex of the pyramid were seen as in deficiency of essential fatty acids (Borland & Jackson, 1931; Moore, 1937). Otherwise the medulla was usually normal in appearance.

The kidneys of rats which had subsisted on diets devoid of vitamin E for 10–15 months showed extensive degeneration and detachment of the epithelial lining of the urinary tubules on microscopic examination. The degeneration was most evident in the convoluted tubules but also affected the loops of Henle and the collecting tubes. In advanced stages of the disease most of the epithelium

of the convoluted tubes had disappeared and in sections of the cortex the glomeruli were seen to be separated by an open network composed of the basement membranes and supporting structures of the degenerated tubules. It was surprising that the animal continued to live with most of its convoluted tubes out of action. The glomeruli showed little change. The glomerular tufts appeared more separated from Bowman's capsule than is usual in sections of the normal kidney, presumably because the volume of the convoluted tubes was diminished. There was no interstitial reaction. The appearance indicated a tubular degeneration only.

Examination of kidneys from rats which had been on the diet deprived of vitamin E for various periods up to 15 months afforded information of the onset and gradual development of the lesion in the kidneys. Degenerative changes were first seen after the animals had been deprived for 3–4 months. At this time the changes were confined to a few convoluted tubes scattered over the cortex and generally near the surface. In these the nuclei of the epithelial cells had lost their usual appearance and were represented by small masses of condensed chromatin. The cells themselves were coarsely granular and irregular in outline and some were detached from the basement membrane. In more advanced stages the nuclei had disappeared and the fused epithelium detached and broken up. Later the epithelium became reduced to an amorphous debris which did not stain with haematoxylin. Finally, the debris disappeared and only the supporting structure remained.

In rats submitted to deprivation for upwards of 10 months the degeneration had become generalized until nearly the whole of the convoluted tubules were destroyed. At the same time many of the tubes of Henle and the collecting tubes were denuded of epithelium. There did not appear to be any concomitant vascular change.

Degeneration was prevented by wheat germ oil concentrate or tocopherol.

Discussion

According to early conceptions the effects of vitamin E deficiency appeared to be confined to the reproductive cycle, the vitamin being necessary for the maintenance of the male sex organs, for the completion of gestation and for the successful suckling of young. Our observations lend support to the wider interpretation of the functions of this vitamin which has recently been gaining ground. Although it is true that the effects of deficiency are first seen in the reproductive organs in both sexes, the lesions finally spread to tissues having no role in reproduction. Prolonged deficiency leads to premature death.

Since the original paper of Goettsch & Pappenheimer (1931) the problem of nutritional muscular dystrophy has attracted much interest among American workers. This condition was first discovered in the course of experiments planned to study the relation between vitamin E and reproduction in the guinea-pig. It was not cured or prevented by wheat germ oil, and was ascribed to deficiency of some unknown factor. Madsen (1936), however, found that

cotton seed oil, a good source of vitamin E, prevented muscular dystrophy in rabbits and guinea-pigs. Morgulis & Spencer (1936) concluded that two factors, one of which is vitamin E, are necessary for protection against muscular dystrophy in the rabbit.

Our observations show that muscular dystrophy occurs in the rat, and is caused by simple vitamin E deficiency. In young paralysed rats muscular dystrophy appears to have been detected first by Olcott (1938). Demole & Pfaltz (1939) found that the paralysis could be prevented by synthetic *dl*-α-tocopherol, and Goettsch & Ritzmann (1939) showed by histological methods that α-tocopherol prevents muscular dystrophy in infant rats. In adult rats muscular dystrophy was described independently by Einarson & Ringsted (1938), Evans *et al.* (1938) and ourselves. The prevention of dystrophy in the skeletal muscles of adult rats by wheat germ oil or concentrate has been clearly established, but results with tocopherol are not yet available.

The brown discoloration of the uterus, which is described in detail in the present paper, was also observed by Barrie (1938), who independently observed muscular degeneration and the deposition of the characteristic yellow granules. Karrer & Demole (1938) also observed the condition, but ascribed it to the effects of resorption. It seems probable that uterine discoloration is an early sign of a pathological condition which later becomes more widespread. The finding of Goettsch & Ritzmann that in the adult rat dystrophy is confined to voluntary muscles is not confirmed.

SUMMARY

1. When virgin female rats were restricted to a diet deficient in vitamin E a brown discoloration of the uterus invariably resulted. This change was seen in a mild form after 3–4 months, and after 12 months presented a striking appearance. The same discoloration was observed in the uteri of rats deficient in vitamin E which had been pregnant. Discoloration could be prevented, but not readily cured, by the administration of vitamin E.

2. Discoloration was also observed in the seminal vesicles of male rats similarly deprived of vitamin E.

3. Both in the uterus and the seminal vesicles pigmentation was confined to the muscular layers, and was due to a deposition of small yellow granules in the smooth muscle cells, some of which were degenerated.

4. After restriction to the deficient diet for periods which varied according to the early nutritional history, male and female rats developed the paresis first reported by Ringsted. The animals became emaciated and in many cases developed skin sores. In animals which were not killed for pathological examination a high rate of mortality occurred.

5. Muscular dystrophy identical with that described by Goettsch & Pappenheimer in the guinea-pig occurred in the hindlimbs of paretic rats. In advanced cases few normal muscle cells remained, the rest being replaced by tubes filled with proliferating nuclei of the sarcolemma.

6. Prolonged restriction to the deficient diet also resulted in a degeneration of the convoluted tubules of the kidney in a high proportion of the animals. The degeneration was unaccompanied by any inflammatory reaction of the interstitial tissues or of the glomeruli.

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EXPLANATION OF PLATE VIII

Fig. 1. A. This discoloured uterus was taken from a virgin rat, about 15 months old, which had received a diet deficient in vitamin E for about 1 year. B. This uterus was taken from a control rat of the same age and history which had received a supplement of 2 drops weekly of a preparation of the unsaponifiable matter of wheat-germ oil.

Fig. 2. A. This uterus was taken from a normal rat receiving an adequate mixed diet, which had been used for breeding purposes. B. This lower uterus was taken from a rat which received a diet deficient in vitamin E and which had undergone pregnancy during routine vitamin E tests over a prolonged period. It was not only discoloured, but permanently enlarged and misshapen.

Fig. 3. The rat in the right partition is a virgin female which received a diet deficient in vitamin E for about 13 months. It is emaciated, rough haired and has paralysed hindlegs. The rat in the left partition is a control animal which has received 3 drops weekly of the unsaponifiable matter of wheat-germ oil.

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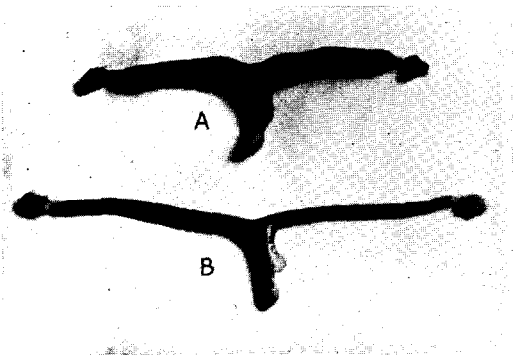

Fig. 1.

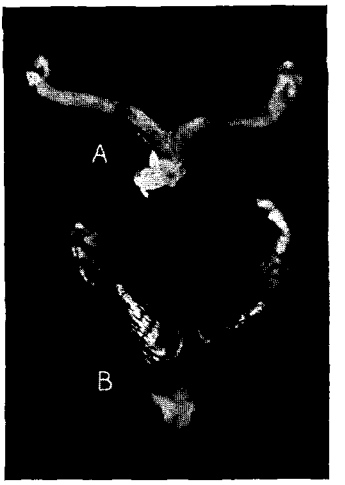

Fig. 2.

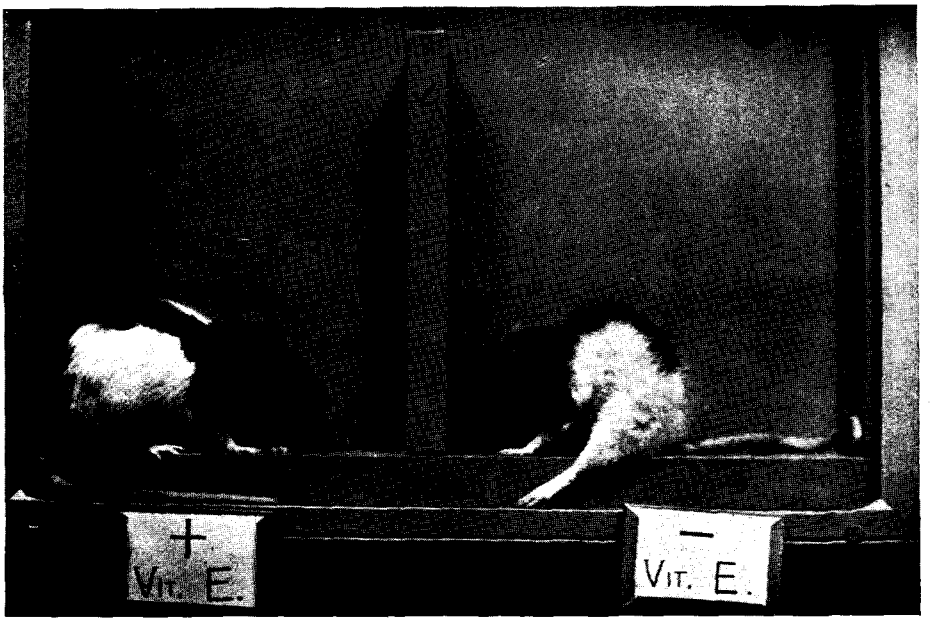

Fig. 3.

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