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CERTAIN NUTRITIONAL DISORDERS OF LABORATORY ANIMALS DUE TO VITAMIN E DEFICIENCY[1]

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In his Welch lecture last October, Dr. Evans (1) presented a masterly review of the current status of the problems centering about the deficiency of vitamin E. It was obviously with some misgiving that I accepted the invitation to speak on a similar topic. However, having yielded to the temptation, I had best concentrate on the pathology of the various disorders which one may with more or less assurance, ascribe to vitamin E deficiency. The chemical aspects of the problems, which are of course the really fundamental ones, I must to my regret, leave to others.

I should like to preface my talk by emphasizing that I am speaking for my colleague, Dr. Marianne Goettsch, as well as for myself. We have been working in close collaboration on these nutritional diseases for the past ten years, and it has been, for me at least, a delightful and profitable association.

I. NUTRITIONAL ENCEPHALOMALACIA OF CHICKS

About ten years ago, Dr. Goettsch (2) and I set out to study the effect of vitamin E deficiency upon reproduction in fowl. We placed day-old chicks upon a simplified diet of skimmed milk powder, casein, lard, cornstarch, cod liver oil, yeast, salts and roughage. They throve nicely for the first three weeks or so, and then began to show a variety of symptoms pointing to some grave disorder of the central nervous system,—tremors, forced movements, head retraction, muscular weakness, somnolence. These symptoms often came on very suddenly, and many of the chicks died; some, however, recovered and as we became more familiar with the disease, we found many instances in which the symptoms were transient or even imperceptible, and yet definite lesions were found when the animals were killed. The incidence of the disease in several thousand chicks on our experimental diet has been about sixty per cent, and we have not succeeded in finding out why all the birds did not succumb. We have tried four different breeds and all are equally susceptible. But as the chicks grow older, the incidence becomes progressively less, and the adult bird is completely resistant.

[1] Delivered at The Blumenthal Auditorium, The Mount Sinai Hospital, New York City, May 14, 1940 as part of the Symposium on Vitamins.

The underlying pathology of this disease proved to be interesting. The cerebellum was most often affected, then the cerebrum, less often the mid-brain and medulla. The essential lesion is an ischemic necrosis—often so extensive as to destroy four-fifths of the cerebellum of one or both hemispheres. Necrosis of ganglion cells, oedema, hemorrhages, and the constant finding of hyaline thrombi in the small vessels—these were the characteristic features, which pointed unmistakably to a circulatory blockage as the cause of the lesions. Ink or dyestuff could not penetrate the affected areas even in the earliest stages. However, we have never been able to determine whether the vascular occlusion was primarily functional or due to the capillary thrombi.

In chicks which survived this acute phase of the disease, reparative changes occurred—gliosis, new growth of blood vessels and reticulum fibers, and calcification. Such spontaneous healing, even in the absence of supplemental treatment with vitamin E, is comparable with the spontaneous recovery which occurs in young rats with muscular dystrophy due to vitamin E deficiency. There appears thus to be a critically susceptible period.

We did not at once recognize this nutritional encephalomalacia of chicks as a manifestation of vitamin E deficiency, in spite of the fact that our experimental diet was purposely lacking in this factor. We were led astray by the observation that a diet of natural foods in which vitamin E had been destroyed by treatment with ethereal ferric chloride after the method of Waddell and Steenbock (3) failed to evoke the disease; also the addition of various foods, supposedly rich in this factor, did not always afford protection. We were further influenced by the current view that vitamin E was concerned primarily, and as was then believed, exclusively, with reproduction. It turned out that we were wrong. The chicks could be regularly protected by a variety of vegetable fats when these were substituted for an equivalent amount of lard in diet 108. The activity was found to reside in the non-saponifiable fraction of the alcoholic extracts, even after removal of the sterols, and a very considerable concentration of the active factor was thus effected. Following the chemical isolation of vitamin E by Evans, Emerson and Emerson (5) in 1936 and its identification as α-tocopherol, it was an obvious experiment to test its efficiency in the prevention of this disease. This has been done by us (6), and independently by Dam, Glavind, Bernth and Hagens (7)—indeed the publicational priority belongs to those workers. It has been found that complete protection may be obtained by the daily administration of 0.2 mg. of either the natural or the synthetic α-tocopherol or the tocopherol acetate. It is of course possible that other tocopherols, or their derivatives may prove effective, but this has not yet been tested. The observation that the protective effect of natural foods against encephalomalacia is not destroyed, even when the anti-sterility factor is rendered

ineffective by ethereal ferric chloride treatment, also demands further explanation. Ni (8, 9) has also obtained partial protection by the addition of 2 per cent donkey skin gelatin (Ah-Chiaco) to an encephalomalacia-producing diet, but the protective factor in this substance has not been chemically defined.

It was interesting to find that this chick disease, manufactured in the laboratory, had its counterpart in the field. "Crazy chick disease," as it is called, had been familiar to New England farmers for a number of years. Its identity, as far as symptoms and lesions are concerned, with the experimental encephalomalacia, was established by Jungherr in 1936 (10), and by others since. There is little reason to doubt that it will be found to be preventable by proper dietary supplement.

Before leaving the subject of vitamin E deficiency in chicks, I should refer to the recent paper of Dam and Glavind (11) on "Alimentary Exudative Diathesis." An extreme subcutaneous edema appeared in some of their chicks, and this they regard as a characteristic manifestation of vitamin E deficiency, indeed, suggesting that it might afford a method of biological assay, since it was preventable by wheat germ oil and by α-tocopherol. In our monograph on "Encephalomalacia" (Bull. 229 of the Stoors Agr. Exp. Station), we have noted the rare occurrence of edema—most often as was the case in the experiments of Dam and Glavind on a low fat diet. It was seen but once on diet 108, and there was no correlation between the generalized edema and the cerebral lesions.

II. NUTRITIONAL MYOPATHY OF DUCKLINGS

When the encephalomalacia-producing diet 108 was tried on ducklings, it produced effects quite different from those found in chicks (12). Clinically, the chief symptom was muscular weakness, so extreme in the last stages, that the animals could not stand erect, or even hold their heads up from the table. Symptoms of brain injury, such as tremors, forced movements, coma, etc., were never observed. And indeed, in the duckling, the brain and other parts of the central nervous system were found to be unaffected. The skeletal muscles, however, showed definite lesions. There was hyaline necrosis of the fibers with rupture and segmentation, followed by a cellular reaction of leucocytes and histocytes, and attempts at regeneration by the activated myoblasts. The lesions are thus practically identical with those produced in guinea pigs, rabbits and young rats by vitamin E deficient diets. Although we have obtained protection with Crisco and partial protection with the non-saponifiable fractions of soy bean oil, the crucial experiment with α-tocopherol has not yet been carried out. This laboratory disease also proves to have its counterpart in the enzoötic muscular dystrophy of ducklings described by Seifried and Heidegger (13) in Bavaria.

III. NUTRITIONAL MYOPATHY OF THE GIZZARD IN TURKEYS

The turkey reacts to vitamin E-deficient diets in a very individual manner. The nervous system and skeletal muscles escape; it appears to be the smooth muscle of the gizzard, as Jungherr first observed, which is peculiarly vulnerable. There appears a patchy hyaline necrosis of the smooth muscle fibers, attended at first by an acute inflammatory reaction, and followed later by fibrosis and attempts at regeneration of the muscle fibers. Dr. Jungherr and I (14) have been able to lower the incidence of the disease by administering soy bean oil or wheat germ oil, but have not as yet tried out α-tocopherol.

Similar lesions have been found in young turkey poults obtained from commercial hatcheries.

IV. NUTRITIONAL MUSCULAR DYSTROPHY IN GUINEA PIGS AND RABBITS

The generalized degeneration of the muscles in these animals, for which Dr. Goettsch and I suggested the term—nutritional muscular dystrophy, was first observed by us in 1931 to develop following an ethereal ferric chloride-treated scorbutic diet supplemented by adequate amounts of orange or tomato juice (15). Since then, it has been studied by a number of other workers—among whom I may mention Morgulies and Spencer (16); Ni (17); Woodward and McKay (18); Chor and Dolhart (19); Shimotori, Emerson and Evans (20); Mackenzie and McCollum (21); Morris (22); and Madsen (23), who have found little difficulty in producing the disease. Although the course, duration and intensity vary considerably in individual animals, their clinical behavior is on the whole quite characteristic. After a period of normal growth, which may range from two weeks to six months or more, there is an abrupt cessation, followed usually by a precipitous decline in weight. The animals become lethargic, and develop increasing muscular weakness to the point of almost complete helplessness, so that they cannot right themselves when placed on their back, and cannot reach their food pans. In this state, they die, and we have never observed spontaneous recovery.

The skeletal muscles throughout the body are found to show extreme lesions, which however are not usually symmetrical, and do not of necessity affect a muscle in its entirety. Both the gross and microscopic appearances depend upon the duration and intensity of the lesions. In the very early and acute stages, which may develop within a few hours, the muscles are somewhat pale and watery, and the contractility is lost. Microscopically, there is extreme hyaline necrosis and fragmentation of the fibers, as well as much interstitial edema. Very soon, however, there is a violent cellular reaction. The necrotic fibers become invaded by polymorphonuclear leucocytes and by histocytes which often fuse to form plasmatic multinucleate masses about the necrotic remnants. These

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may become calcified. Particularly in young animals, the muscle nuclei, which escape destruction, early become activated. They divide mitotically, arrange themselves in rows; new myofibrils are formed on the surface; the cytoplasm which at first stains purplish, becomes red as the myohemoglobin is regenerated. This regeneration is sometimes extraordinarily active, even while the degenerative alterations are in full blast.

The dystrophic changes are not always so fulminating as I have depicted them; and the disease may run a chronic course. In such animals, comparatively few fibers are destroyed at any one time, but their gradual loss and replacement by fat and fibrous tissue brings about a picture which is identical with that of an advanced case of human muscular dystrophy. Such animals may survive for many months, dying finally of inanition or of a terminal pneumonia. There are several points in the pathology which I should like to stress. One is the excellent preservation of neurites and end-plates in the midst of the necrotic muscle cells (21). With Dr. Wolf's assistance, we have studied the brain and spinal cord quite thoroughly in some of our animals, and have seen no alterations which seemed to be of significance.[2] The histological evidence, therefore, favors a primary muscular lesion, rather than a neural one.

Another point is the striking selectivity of the lesions for the skeletal muscles. Madsen (23), working in our laboratory, found degenerative changes in the heart muscle of a few rabbits and guinea pigs, but I am not wholly convinced that these lesions are referable to the vitamin E deficiency. They are certainly not a usual finding in this disease. The smooth muscle is never affected.

Most of the animals die before the age of sexual maturity, and we have not been able to study the effect of vitamin E deficiency upon reproduction in the rabbit or guinea pig. One of our rabbits, however, gave birth to two young while she was still in the incipient stage of the disease as shown by biopsy. The young were scrawny and weak, and survived but a day. Their muscles were found to show extreme degeneration, so that the development of the disease in utero was certain, and the normal transference of vitamin E to the embryo through the placenta may be assumed.

Since the preliminary report of this observation in 1936 (25), we have been able to study 20 young born of mothers maintained on diet 48 in which 8 per cent of soy bean oil was substituted for the lard in diet 11. This sufficed to protect the mothers against muscle dystrophy for periods up to 958 days. It was not sufficient, however, to prevent the development of muscle dystrophy in the offspring during late intra-uterine or early post-natal life. Sixteen of the twenty young, born dead or surviving less

[2] Ekblad and Wohlfart (Ztsch. f. I. Ges. Neurol. u. Psych.: 168: 145, 1940) have recently described sclerotic changes in the ganglion cells of the spinal cord. These have not been present in our material.

than 5 days, showed lesions of varying intensity in the muscles. The remaining four with normal muscles were all first litter animals.

An interesting feature of the disease in newborn rabbits is the accompanying edema of the subcutaneous tissue and intramuscular connective tissue, which is perhaps analogous to the "exudative diathesis" regarded by Dam and Glavind as a manifestation of vitamin E deficiency in the chick.

There is also an analogy to the muscular dystrophy of young rats born of mothers partially deprived of vitamin E, with this difference that in the rabbits, the disease is present at birth, while in the rats it appears only at the end of lactation.

What is the evidence that this muscle disease of rabbits and guinea pigs is really a manifestation of vitamin E deficiency? This question was discussed very thoroughly by Dr. Evans in his Welch lecture. The evidence in favor of this view is accumulating. In our original paper, Dr. Goettsch and I thought that we could eliminate the lack of vitamin E as a factor in the causation of the disease, since daily doses of approximately 200 mg. of tested wheat germ oil failed to protect guinea pigs. In addition guinea pigs and rabbits upon the diet which had not been treated with ethereal ferric chloride, and contained vitamin E in amounts adequate for normal reproduction in rats, eventually developed dystrophy. In the light of subsequent work, it would seem that the dosage, upon the particular diet used (containing lard and cod-liver oil) was probably inadequate. Mackenzie and McCollum (21), using the creatin excretion as a criterion of the disease have obtained curative effects with α-tocopherol; Morris (22), with doses of 18 to 25 mg., obtained definite symptomatic cures; Shimotori, Emerson, and Evans (20) effectively prevented the disease in guinea pigs over a period of 200 days by supplementing the diet with 3 mg. of synthetic α-tocopherol on alternate days. Dr. Goettsch and I have also succeeded in producing remissions and in a few instances permanent cures in guinea pigs by feedings or injections of 20 to 25 mg. of synthetic α-tocopherol. The progress of the disease in our animals was followed by repeated muscle biopsies, and we were amazed to find that severe lesions may completely disappear within a week following the injection or feeding of a single dose. The evidence then supports the view that vitamin E deficiency is the essential thing in the causation of this disease, and does not substantiate the contention of Morgulies (24) that there is an additional water soluble factor (B_4?) concerned.

The point of view advocated by Madsen, McCay and Maynard (27) that the toxicity of cod-liver oil for the herbivora is the essential factor in the production of muscular dystrophy, can not, we think be maintained in the face of Cummings and Mattill's (28) demonstration that cod-liver oil brings about the oxidative destruction of vitamin E. The more recent findings of Davis, Maynard and McCay (29) show that the addition of

3 per cent cotton-seed oil to a synthetic dystrophy-producing diet prolongs and in some cases prevents the disease. This finding can also be explained by the vitamin E content of the vegetable oil.

A rather instructive illustration of the effect of cod-liver oil in precipitating muscular dystrophy was brought to our attention by Dr. Leonard Goss of the Bronx Zoological Garden (30). Four tree-kangaroos were placed on exhibition at the World's Fair, and to make them especially presentable, they were given large amounts of fish liver oil. They sickened, and three of them died, with extreme muscular dystrophy. The third one was brought back to the Bronx Zoo, but continued to show muscular weakness in spite of the withdrawal of fish liver oil from the diet. He was then given α-tocopherol and made a rapid and spectacular recovery.

V. MUSCULAR DYSTROPHY IN YOUNG RATS

This chapter of the story begins with the report of Evans and Burr (31) in 1928, that the offspring of female rats, partially depleted of vitamin E, often became more or less completely paralysed towards the end of lactation. Some of their rats died, others recovered, with or without residual weakness. But it was not until the publication of Olcott's paper in 1936 (32) that the pathology underlying these symptoms was made clear. Olcott found widespread necrosis of the skeletal muscles—lesions essentially like those in the more acute phases of the muscular dystrophy of rabbits and guinea pigs. We have confirmed and perhaps amplified Olcott's observations (32), as have Telford, Emerson and Evans (34) The disease often develops with almost explosive suddenness; and what to us seems very remarkable, it appears to be self-limited if the young rats survive. Even without any vitamin E supplement, healing of the muscle lesions occurs with astonishing rapidity, so that after a week, little trace of the original devastation can be found. This is one of the interesting problems in connection with this curious disease that remains to be investigated.

As was the case with the chicken encephalomalacia, the symptoms are not always a reliable criterion. We frequently find extensive muscle lesions in rats killed on the 24th or 25th day. These rats have shown none of the usual symptoms such as clenching of the paws, rough fur, bloody crusts about the eyes, and paresis.

Dr. Goettsch and Dr. Ritzmann (35), modifying Evan's original procedure slightly, have induced the muscle disease in a high percentage of rats. There appear, however, to be individual differences in the amount of vitamin required by the mother rat to protect her children against the disease, and these constitutional differences which may conceivably have a bearing upon the incidence of muscle dystrophy in humans, are being studied further.

Although our own studies have been restricted almost entirely to young

rats, others have found lesions both of the muscles (Knowlton and Hines) (36) and of the spinal cord (Einarson and Ringsted) (37) in older rats maintained for several months on a vitamin E-deficient diet. The studies of the Danish observers are of particular interest inasmuch as the degeneration of the pyramidal tracts and anterior cells, as well as of the dorsal sensory tracts, offer a resemblance to the lesions of amyotrophic lateral sclerosis in man. In two of a number of our older rats, Dr. Wolf has found similar changes. The therapeutic results recently reported by Dr. Wechsler (38) and Bicknell (39) in England at least offer the hope that the analogy between the rat and the human disease is not a superficial one.

It is now well established through the work of Evans and Burr (31), of Morelle (40), of Barrie (41), of Demole and Pfaltz (42), of Goettsch and Ritzmann (35) and of Knowlton, Hines and Brinkhous (43) that this muscular dystrophy of young rats is effectively prevented by wheat germ oil, and by α-tocopherol, either natural or synthetic. A single dose of 0.5 mg. administered on or before the 17th day regularly confers protection. Oil of wheat germ treated with ethereal ferric chloride, was still anti-dystrophic in spite of the fact that 20 gm. failed to prevent resorption in vitamin E depleted rats, and this discrepancy invites further study.

In the hope of learning something further of the pathogenesis of the muscle lesions, we have during the past winter, performed some rather interesting though childishly simple experiments (44).

The sciatic nerve to the left leg was resected and the histological lesions of the gastrocnemius muscle on the two sides were compared. It was found to our surprise that the dystrophic lesions did not develop in the muscle deprived of its nerve supply when the operation was performed at any time between the 5th and the 17th day. When, however, the nerve was cut on the 18th day, the protection was no longer effective—either because the irritability of the distal segment persisted for a day or two, or because the changes initiating the muscle necrosis were already under way, although there were at the time no visible symptoms or gross lesions (table 1).

These results seemed to inculpate some obscure nervous influence in the production of the lesions. We have had the thought that the necrosis might be the result of angiospasm. The persistence of a shell of intact fibers on the surface of the muscle suggested that they might have been spared because of an additional blood-supply from the fascial vessels. Loss of sympathetic innervation through section of the sciatic nerve might then have inhibited the vasoconstriction causing the necrosis.

This pretty idea received a rude shock when we found that section of the Achilles tendon leaving the nerve supply intact, was equally effective in protecting the gastrocnemius (table 2). It is, therefore, not the loss of nerve supply *per se* which is essential, but rather the loss of muscle tonus, that affords protection. We have found further that mere in-

activity of the muscle is not sufficient to prevent the lesions. We placed one hind limb in a molded copper splint completely immobilizing the knee and tibio-tarsal joints in the extended position. Since the mother rat resolutely opposed this procedure, and tore off the splints, it was necessary to feed the young rats on skimmed milk with a dropper. The splinting

TABLE 1

Effect of section of left sciatic nerve upon muscular dystrophy of young rats on vitamin E deficient diet

SERIAL NUMBER	LITTER NUMBER	AGE AT OPERATION	AGE AT DEATH	LESIONS		
				R. gastrocnemius	L. gastrocnemius	Other muscles
		days	*days*			
1	234 a	5	21	++++	−	++++
2	199 a	7	22	++++	−	++++
3	200 a	12	19	++++	−	++++
4	200 c	12	20	++++	−	++
5	189 a	15	16	±	−	−
6	189 b	15	18	+ (?)	+++ (?)	++++
7	196 a	15	20	+++	−	+++
8	196 b	15	20	+++	−	+++
9	196 c	15	24	++++	−	+++
10	196 e	15	24	±	−	−
11	196 g	15	22	+++	−	−
12	196 h	15	22	+++	−	−
13	196 i	15	23	+	−	−
14	218 a	15	24	±	−	+
15	218 b	15	23	+++	−	+++
16	218 c	15	24	+++	−	+++
17	218 d	15	23	+++	−	+++
18	240 a	16	21	++++	+	++++
19	240 b	16	21	++++	−	++
20	217 a	17	19	++++	−	+++
21	217 b	17	22	++++	−	+++
22	216 a	17	25	++++	−	++++
23	224 a	18	20	+++	+++	+++
24	224 b	18	20	+++	+++	+++
25	224 c	18	20	+++	+++	+++
26	224 d	18	25	+++	+++	+++
27	224 e	18	25	−	+++	+++
28	244 a	18	20	++++	++++	++++
29	244 b	18	25	++++	−	−

did not protect, and lesions were found on both sides. The effect of transection of the spinal cord at the lower dorsal or upper lumbar levels was also studied in a fairly large series, but the effects as regards the muscles of the lower extremities were not consistent, probably because of the varying extent of the destruction of the cord below the level of the section.

The interpretation of such experiments is not easy, and it is obvious that much further work must be done before we can even guess at the precise rôle which vitamin E plays in muscle physiology. That the denervated or tenotomized muscle is less sensitive to the lack of vitamin E than the functionally active muscle, seems proven. But we do not know why, and have not been able, as yet, to formulate a plausible hypothesis.

One is tempted to conclude a lecture of this sort with some sweeping generalizations. But it is pretty obvious that we are only at the beginning

TABLE 2

Tenotomy of left gastrocnemius

SERIAL NUMBER	LITTER NUMBER	AGE AT OPERATION	AGE AT DEATH	LESIONS		
				R. gastrocnemius	L. gastrocnemius	Other muscles
		days	*days*			
1	239-4	10	24	++++	±	++++
2	239-5	10	24	+	−	±
3	239-6	11	24	++++	−	++++
4	211-5	14	19	++	−	+++
5	216-1	16	21	++++	++++	++++
6	216-6	16	21	++++	−	++++
7	216-4	16	22	++++	−	++++
8	254-2	17	25	++	−	+++
9	254-4	17	25	++++	−	++++
10	255-5	17	25	++++	−	++++
11	254-7	17	25	++++	−	+++
12	258-6	17	25	++++	−	++++
13	258-7	17	21	++++	−	++++
14	258-8	17	24	++++	−	++++
15	258-9	17	25	++++	−	++++
16	258-10	17	25	++++	−	++++
17	234-1	17	23	++	−	++++
18	234-2	17	25	++++	−	++++
19	234-4	17	18	++	−	++++
20	234-5	17	24	++++	±	++++

of our knowledge as to the fundamental rôle which the tocopherols and related substances play in nutrition. As to the applicability of results obtained with laboratory animals to problems of human disease, one can make no forecasts, but the work has gone far enough, it seems to me, to justify a cautious empiricism. The fact that a partial deficiency of vitamin E in the mother may manifest itself only in the offspring, seems to me to be one of the most significant lessons that one can draw from this work. May not similar things happen in human diseases, and help to explain the supposed hereditary or familial character of certain nervous and muscular disorders?

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15	218 b	15	23	+++	−	+++
16	218 c	15	24	+++	−	+++
17	218 d	15	23	+++	−	+++
18	240 a	16	21	++++	+	++++
19	240 b	16	21	++++	−	++
20	217 a	17	19	++++	−	+++
21	217 b	17	22	++++	−	+++
22	216 a	17	25	++++	−	++++
23	224 a	18	20	+++	+++	+++
24	224 b	18	20	+++	+++	+++
25	224 c	18	20	+++	+++	+++
26	224 d	18	25	+++	+++	+++
27	224 e	18	25	−	+++	+++
28	244 a	18	20	++++	++++	++++
29	244 b	18	25	++++	−	−

did not protect, and lesions were found on both sides. The effect of transection of the spinal cord at the lower dorsal or upper lumbar levels was also studied in a fairly large series, but the effects as regards the muscles of the lower extremities were not consistent, probably because of the varying extent of the destruction of the cord below the level of the section.

The interpretation of such experiments is not easy, and it is obvious that much further work must be done before we can even guess at the precise rôle which vitamin E plays in muscle physiology. That the denervated or tenotomized muscle is less sensitive to the lack of vitamin E than the functionally active muscle, seems proven. But we do not know why, and have not been able, as yet, to formulate a plausible hypothesis.

One is tempted to conclude a lecture of this sort with some sweeping generalizations. But it is pretty obvious that we are only at the beginning

TABLE 2

Tenotomy of left gastrocnemius

SERIAL NUMBER	LITTER NUMBER	AGE AT OPERATION	AGE AT DEATH	LESIONS		
				R. gastrocnemius	L. gastrocnemius	Other muscles
		days	*days*			
1	239-4	10	24	++++	±	++++
2	239-5	10	24	+	−	±
3	239-6	11	24	++++	−	++++
4	211-5	14	19	++	−	+++
5	216-1	16	21	++++	++++	++++
6	216-6	16	21	++++	−	++++
7	216-4	16	22	++++	−	++++
8	254-2	17	25	++	−	+++
9	254-4	17	25	++++	−	++++
10	255-5	17	25	++++	−	++++
11	254-7	17	25	++++	−	+++
12	258-6	17	25	++++	−	++++
13	258-7	17	21	++++	−	++++
14	258-8	17	24	++++	−	++++
15	258-9	17	25	++++	−	++++
16	258-10	17	25	++++	−	++++
17	234-1	17	23	++	−	++++
18	234-2	17	25	++++	−	++++
19	234-4	17	18	++	−	++++
20	234-5	17	24	++++	±	++++

of our knowledge as to the fundamental rôle which the tocopherols and related substances play in nutrition. As to the applicability of results obtained with laboratory animals to problems of human disease, one can make no forecasts, but the work has gone far enough, it seems to me, to justify a cautious empiricism. The fact that a partial deficiency of vitamin E in the mother may manifest itself only in the offspring, seems to me to be one of the most significant lessons that one can draw from this work. May not similar things happen in human diseases, and help to explain the supposed hereditary or familial character of certain nervous and muscular disorders?

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