Nutritional Muscular Dystrophy in Monkeys Receiving a Diet Deficient in Both Vitamins B_6 and E

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Several years ago we observed unmistakable signs of vitamin E deficiency in monkeys receiving a diet containing, among other ingredients, polished rice and whole wheat. However, the diet contained a small amount of tocopherols and was therefore not well suited to the study of vitamin E deficiency. On this diet, muscular weakness and the chemical evidences of vitamin E deficiency developed only after two or three years.[2]

Recently we[3-5] have obtained evidence on lower animals suggesting a metabolic interrelationship between vitamin B_6 and vitamin E. Thus, when weanling albino rats were given a diet deficient in both vitamins B_6 and E they developed a leukocytosis characterized by greatly increased peripheral neutrophil counts. Supplementation of the diet with either of these vitamins prevented the increase. The vitamin B_6-deficient rats exhibited a slight lymphopenia which was not affected by vitamin E. None of the rats developed an anemia during the period of the experiment. Some of the data are shown in Table I.

In such experiments the rats deficient in vitamin E alone grew nearly as well as those receiving both vitamins. On the other hand, rats deficient in vitamin B_6 grew almost as poorly as those deficient in both vitamins.[4] Both vitamins, however, influenced the excretion of creatine and allantoin. The deficient (basal) group excreted large amounts of creatine, and *either* vitamin prevented this creatinuria. Similarly, the rats deficient in both vitamins excreted increased amounts of allantoin, and this was prevented by either vitamin B_6 or vitamin E.

TABLE I

Effects of Vitamins E and B_6 on Peripheral Leukocyte Counts in Rats
(Cells in thousands per microliter)

Diet	Total leukocytes	Lymphocytes	Monocytes	Neutrophils	Eosinophils
Basal (deficient)	18.4	6.9	0.6	10.5	0.4
" + E	8.5	5.7	0.1	2.4	0.1
" + B_6	10.0	7.2	0.4	2.2	0.2
" + E + B_6	10.8	8.8	0.4	1.5	0.1

In view of this apparent interrelationship between vitamin B_6 and vitamin E in the metabolism of the rat, it seemed possible that such a double deficiency might accelerate the production of vitamin E deficiency in the monkey. Consequently, we have subjected a number of young rhesus monkeys to a diet of purified materials which is deficient in both vitamins.

MATERIALS AND METHODS

The composition of the diet is shown in Table II.

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TABLE II

Composition of Diet Deficient in Vitamin E and B_6

(Daily allowance per monkey)			
	grams		*mg*
Casein, vitamin-free	18	Riboflavin	0.5
Lard	8	Calcium pantothenate	2.0
Salt mixture	4	Nicotinic acid	2.0
Choline chloride	0.1	Menadione	0.44
Inositol	0.1	Folic acid	0.5
Corn starch	46.2	Thiamine chloride	0.5
Sucrose	21.6	Ascorbic acid	20.0
Baking powder	1.5		
Cod liver oil	3.0		

The casein furnishes essential amino acids, lard and cod liver oil supply essential fatty acids, the salt mixture supplies all of the required inorganic elements, and the starch and sucrose yield energy. All of the vitamins believed to be required by the monkey are included, except vitamins B_6 and E. The diet ingredients, except the cod liver oil, thiamine chloride, and ascorbic acid, are mixed together with enough water to make a thick batter and then cooked in a moderate oven in the form of a crisp wafer. The baking powder gives a light texture to the wafers. The cod liver oil, thiamine chloride, and ascorbic acid are placed on the wafers just before feeding. The monkeys pick up the wafers and eat them greedily.

The animals are housed in steel metabolism cages and given this diet and water once daily. The cages are equipped with funnel-shaped stainless steel trays for the collection of urine. The 24-hour urine specimens were analyzed for creatine, creatinine, and allantoin.

At intervals of one week, or oftener, blood was drawn from an ear vein of each monkey and examined by standard hematological techniques for erythrocytes, reticulocytes, hemoglobin, hematocrit, total white cells, and differential white cell counts.

RESULTS

After about 10 months on this diet the animals developed a progressive muscular weakness, which developed first in the hind quarters and progressed to the front limbs. In the extreme stage the animals experienced difficulty

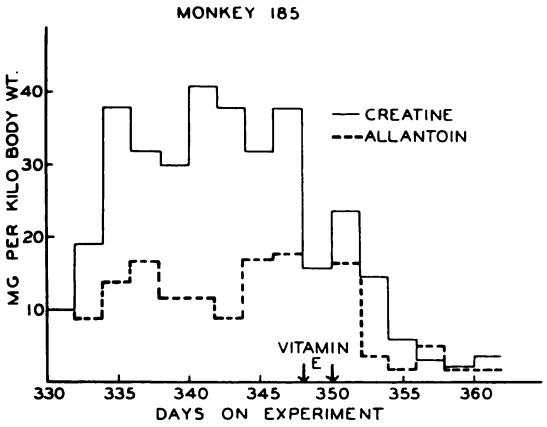

Fig. 1. Creatine and allantoin excretion by a deficient monkey.

in getting to a sitting position. They tired easily and had difficulty in breathing. Even in the most extreme stage of the deficiency, however, they were able to eat.

Accompanying this muscular dystrophy were changes in the cytology of peripheral blood and in the amounts of certain nitrogenous constituents of the urine. Analyses of urine specimens of a typical animal are shown in Figure 1.

It will be seen that on the 330th day there was a sharp increase in creatine excretion and a moderate increase in the output of allantoin. This continued until the diet was supplemented with vitamin E (α-tocopherol) on the 348th and 350th days. This treatment with vitamin E was followed by an abrupt reduction in the output of creatine and allantoin. Both of these urinary constituents fell to normal levels.

Figure 2 shows in graphic form the *average* output of creatine, creatinine, and allantoin during the period of obvious muscular dystrophy. The diagonally striped bars represent the output of a normal animal, while the solid bars represent the output of a dystrophic animal. It will be seen that the deficient monkey showed a 30-fold increase in creatine excretion, and a 2.5-fold increase in allantoin excretion, but the output of creatinine was only one-half that of the normal animal.

Figure 3 shows the peripheral blood cell picture of a deficient animal. Normally, the total white blood cell count of a young monkey is between 10 and 20 thousand cells per microliter. The dystrophic monkeys consistently

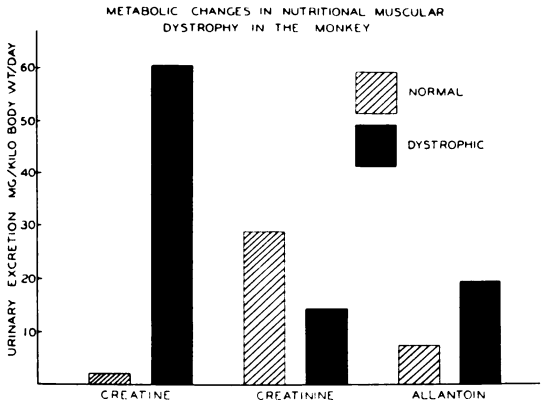

Fig. 2. Average daily excretion of creatine, creatinine, and allantoin, by a dystrophic monkey compared to a normal.

showed a moderate increase, frequently to values above 20 thousand per microliter. On the 348th and 350th days this animal was given a dose of vitamin E. This treatment was followed by a moderate decrease in the total white blood cells, a sharp decrease in granulocytes, and a moderate increase in lymphocytes.

The lower part of the figure shows the responses of the red cells of the animal. There was a mild anemia, characterized by an erythrocyte count of less than 3 million per microliter, a reduction in hemoglobin to approximately 8 grams per 100 ml of blood, and a reduction in hematocrit to 25 per cent.

Following the administration of two doses of 20 mg of α-tocopherol each, as shown by the arrows, there was a sharp increase in reticulocytes to 12 per cent, and a gradual return of erythrocytes, hemoglobin, and hematocrit to, or at least toward, normal levels.

The effect of vitamin E deficiency on the circulating blood cells is shown in Figure 4. In this bar graph the height of the bar represents the percentage of normal for each of the blood constituents. At the point in the experiment when the animals were definitely dystrophic the erythrocytes were reduced to approximately 50 per cent of normal, the hemoglobin to about 70 per cent of normal, and the hematocrit to 75 per cent of normal. However, the neutrophils were 250 per cent of normal, but the lymphocytes only 50 per cent of normal levels.

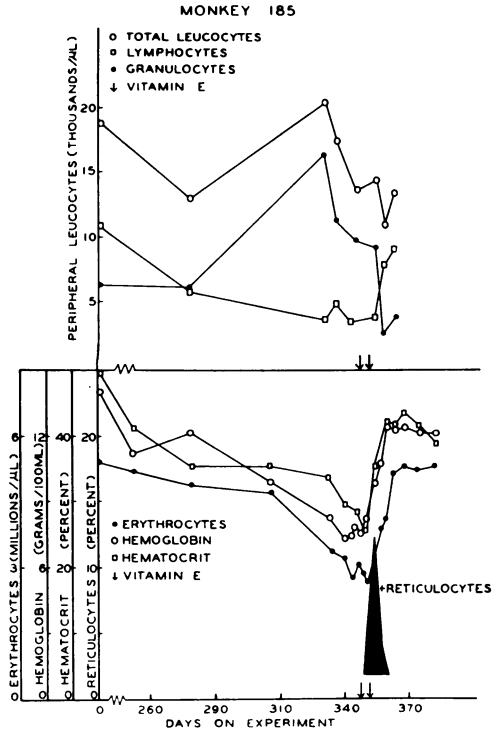

Fig. 3. Blood picture of a deficient monkey.

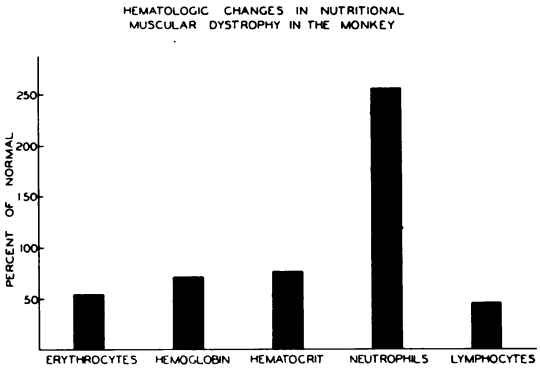

Fig. 4. Graph showing average blood counts of a deficient animal in comparison with normal values.

Muscle biopsies were taken of a dystrophic and a normal monkey. Histopathologic sections demonstrated clear evidence of muscle fiber degeneration in the deficient monkey as compared with the normal.

Moving pictures were shown which presented successively a normal monkey and then three stages in the development of dystrophy and its cure with α-tocopherol. "The normal young rhesus monkey is extremely active. When

the cage door is opened and the handler attempts to catch him, he jumps out of the cage and springs from the top of one cage to another, never seeming to tire. The second scene shows the earlier stages of dystrophy. When the cage door is opened the monkey attempts to hide in the corner. When removed from the cage he is able to crawl up the outside of the cage with difficulty. In the more extreme stage of the deficiency he has great difficulty in moving at all. When laid on his side on the floor he is able to struggle to a sitting position by pushing himself into a sitting position with his arms. He is able to crawl only with difficulty and his attempts to climb into his cage fail completely. However, 11 days later, following treatment with 40 mg of α-tocopherol in divided doses, his muscle strength has returned somewhat, and he is able to climb up the side of a cage, and into his cage with considerable agility."

SUMMARY

These experiments indicate that the young rhesus monkey is susceptible to vitamin E deficiency, showing a syndrome characterized by progressive muscular weakness, increase in urinary output of creatine and allantoin, but a reduction in urinary creatinine, progressive anemia, and leukocytosis. All of these physical, chemical, and cytological changes respond to the administration of α-tocopherol. We do not yet know what part the deficiency of vitamin B_6 may have in the development of the syndrome.

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DISCUSSION

DR. R. E. OLSON (Pittsburgh, Pa.): I was very fascinated by Dr. Day's paper because it represents the study of a multiple deficiency disease in which vitamin E is one of the absent nutrients. In our laboratory, we have undertaken a study of the biochemical pathogenesis of still another multiple deficiency disease in which vitamin E is one of the absent nutrients and cystine is the other. This deficiency disease is known as dietary hepatic necrosis and has been described by various investigators, including Daft of the National Institutes of Health, György of the University of Pennsylvania, Himsworth of the University of London, and Klaus Schwarz, currently of the National Institutes of Health.

We have found that animals fed diets deficient in these nutrients demonstrated marked changes in their hepatic carbohydrate metabolism prior to the onset of gross pathologic lesions. Although certain enzymes of the Krebs tricarboxylic acid cycle were found intact, the overall oxidation of pyruvate and acetate was found to be depressed. We found, further, that some of the coenzymes catalyzing reactions in the cycle were depressed, one of the most conspicuous being coenzyme A. We reasoned initially that this finding bore an important relationship to the pathogenesis of the disease, since dietary cystine probably served as the precursor for the thioethanolamine moiety of coenzyme A, and, in the absence of vitamin E, might become limiting for coenzyme A synthesis. We reasoned, further, that this limitation in coenzyme A synthesis could lead to biochemical ischemia and liver necrosis.

It has turned out, however, that the problem is not quite as simple as initially thought. We have studied the levels of coenzyme A in animals fed a diet low in organic sulfur (cystine and methionine) with and without supplemen-

tary vitamin E. In both cases, the coenzyme A content of liver is markedly reduced, in most instances there being no significant differences between the values for hepatic coenzyme A in animals receiving and not receiving vitamin E, even though only those on vitamin E-deficient diets go on to develop hepatic necrosis. If now, however, one attempts to define more functionally the metabolism of coenzyme A by a study of the rate of incorporation of S^{35}-labeled cystine into this coenzyme in these animals, one finds a marked difference in the animals on a necrogenic diet as compared to animals receiving the same diet with added vitamin E. The rate of incorporation of S^{35} from cystine into liver coenzyme A in the deficient group is approximately 33 per cent of that observed in the animals which are given vitamin E.

What, precisely, these data mean in terms of the relationship between vitamin E and sulfur-amino acid metabolism is not clear at this time. It may be related to the overall metabolic activity of the coenzyme which is reflected by an increased turnover rate of one of its components. This, in turn, may result indirectly from an action of vitamin E not presently defined. On the other hand, it may represent a defect in the synthesis of coenzyme A from organic sulfur precursors by the vitamin E-deficient rat which is matched by catabolic rates in such a way that the level is not affected.

The reason I felt that these data might be of interest to this group in connection with Dr. Day's paper is that we know the nutrient with which he has been concerned in addition to vitamin E, namely vitamin B_6, is also concerned in an important way with the metabolism of sulfur-amino acids. Specifically, pyridoxal phosphate functions as a coenzyme in the desulfuration of cysteine and the decarboxylation of cysteinesulfinic acid. It might be that an important function of vitamin E is to regulate sulfur-amino acid metabolism and that simultaneous deficiencies of cystine and/or vitamin B_6 result in additional widespread pathology.