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Experimental Production of Arteriosclerosis in Rhesus Monkeys by Deficiency of Pyridoxine Hydrochloride (Vitamin B-6) and Essential Fatty Acids

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ABSTRACT

Experimental arteriosclerosis that closely resembles human arteriosclerosis was produced by a deficiency of pyridoxine and essential fatty acids in monkeys subjected to a synthetic diet. A detailed analysis of the pathological changes in the various organs disclosed that the changes produced differed in severity as follows: deficiency of both pyridoxine and essential fatty acids produced the most severe changes, then pyridoxine deficiency alone and then essential fatty acids deficiency alone.

In view of the data obtained, attention is called to the importance of vitamin B-6 in metabolism, particularly in its relation to protein metabolism and its possible connection to the pathogenesis of human arteriosclerosis. The importance of essential fatty acids also cannot be minimized. Deficiency in both of these two factors certainly appears to be related to the enhancement of arteriosclerosis in monkeys.

Despite almost limitless investigations and reports, the exact etiology and pathogenesis of arteriosclerosis in the human is still unknown. There has been much accumulated evidence however, to suggest or to incriminate certain factors in the causation of arteriosclerosis, but none of this is universally accepted. One of the more attractive approaches, both clinically and experimentally, has been to deal with dietary factors.

A new way to re-explore the metabolic and structural defects resulting from single vitamin deficiencies was opened by Rinehart and Greenberg in 1949 by adapting the rhesus monkey (Macaca mulatta) to a synthetic diet. During the course of their long-term investigations, they reported the occurrence of degenerative vascular lesions in rhesus monkeys subjected to pyridoxine deficiency, and pointed out that these degenerative lesions simulated very closely those of arteriosclerosis in man.

The present investigation is designed to explore the relationship of essential fatty acid deficiency and of pyridoxine deficiency to vascular disease, subjecting animals to single and combined deficiencies of essential fatty acids and vitamin B-6. Particular attention is paid to reporting pathological findings of various organs in detail, with particular emphasis on the vascular changes.

MATERIALS AND METHODS

Three groups of healthy rhesus monkeys weighing from 2.5 to 3.0 kg were used. The first group of animals received a synthetic diet deficient in pyridox-

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ne, the second group was given a diet deficient in essential fatty acids, and the third received a diet deficient in both pyridoxine and essential fatty acids. The experiments also carried a group of control animals.

The basic diet of the experimental animals was a modification of the M-3 diet of Waisman and associates, and consisted of powdered sucrose 73%, vitamin-test casein (Labco) 18%, Hawk and Oser salt mixture 4%, and corn oil 2%. The dry ingredients were thoroughly blended, granulated, and compressed into 2 gm tablets after the addition of 1% calcium stearate. These tablets were fed ad libitum. Each monkey received one vitamin tablet daily containing the following thiamine, 0.5 mg; riboflavin, 1 mg; nicotinic acid, 5 mg; calcium pantothenate, 3 mg; ascorbic acid, 25 mg; p-aminobenzoic acid, 100 mg; choline dihydrogen citrate 100 mg; inositol, 100 mg; and biotin, 10 micrograms, plus sufficient powdered sucrose to make a tablet weighing 1.5 grams. Control monkeys also received either 1 mg pyridoxine hydrochloride daily or 3.5 mg twice a week (on a sugar cube). In addition, each monkey received by mouth 10 drops of a vitamin A and D concentrate (containing 100,000 units of vitamin A and 10,000 units of vitamin D) and 5 drops of mixed natural tocopherols (containing 340 mg/g) weekly.

The first group of animals received the above diet mixture except for pyridoxine, the second group was given the diet without corn oil, and the third received the diet deficient both in pyridoxine and corn oil.

The animals either died or were sacrificed after 1½ months to 25 months. Gross features of the various organs were recorded at autopsy, and tissues were fixed in 10% neutral formalin. Histologic sections were stained mainly with hematoxylin and eosin. In addition, the following histochemical stains were made in order to demonstrate the specific vascular changes: aldehyde-fuchsin, colloidal iron, and fat stains.

RESULT

The pyridoxine-deficient group of animals showed gross changes essentially similar to those observed by previous investigators. The animals continued to eat well and gained weight for 2 to 3 weeks, after which the food consumption began to fall off and the animals lost weight. The weight loss was a gradual one in most instances. The animals also showed diminished vigor and became sluggish and jumpy. The hair became thinner and lighter, and late in the course of the experiment most had developed some fissuring of the epithelium of the palms of the hands and feet. All developed a moderate leukopenia and anemia. The essential fatty acid-deficient group of animals showed definitely retarded growth as compared to the control animals, but there was no significant change in outward appearance from the control animals. However, the animals in the combined deficiency group certainly failed earlier than the animals subjected to simple vitamin B-6 deficiency or to simple essential fatty acids deficiency.

The histological changes observed in all the experimental animals are summarized in Table 1. As seen in Figures 1-6, there occurred prominent vascular sclerosis closely resembling that of human arteriosclerosis in arteries of various sizes.

Group	Monkey No.	Exper. Period (Mos.)	Aorta	Muscular Artery	Liver	Other
Controls (normal)	147	7½	0	0		
B-6 Defic.	145	7½	0	Early 1 esions	Fatty	Parasites
	146	10½	2+	3+	Fatty Abscess	
	188	5½	0	+	Fatty	Parasites
	189	7-1/3	+	0	Fatty	
EFA Defic.	150	7-3/4	0	2+Coronary		
	152	25½	0	+Renal	Abscess	
	154	25½	0	2+		

B-6-EFA	160	8	+	+ to 4+	Cirrhosis	Parasites
Defic.	162	15	±	2+	//	Calc. Parasites in adrenal.
	181	11	0	2+	// 3+	Islet lesion? Pneumonia.
	182	1½	0	0	Fatty	Parasites
	183	11	+	0	Early Cirrhosis	
	184	4	0	+	Fatty	Parasites

The Pyridoxine deficient group: Animals No. 145, No. 146, No. 188, and No. 189.

The prominent changes in arteries of this group of animals were seen in the mucinous ground substance. This change was characterized by swelling which was most marked in the intima, but in the more severe lesions there was also a swelling of the ground substance of the media. Increased accumulation of mucinous material caused marked thickening of the intima. Proliferation of the intimal cells was also noted.

The sclerotic alterations of the arteries in this group of animals were widely distributed, but the site of predilection seemed to correspond very closely to that in the human. Animal No. 145 showed the aorta, coronary artery and renal arteries to be relatively free of sclerotic change, but muscular arteries showed early changes in the intima. The liver showed slight fatty changes. Parasites were identified in the intestine in this animal.

Animal No. 146 showed remarkable sclerotic intimal changes in the aorta as seen in Figure 1. Medium-sized muscular arteries also showed intimal thickening due to accumulation of mucinous material and proliferation of intimal cells (Figure 2). The liver showed fatty changes and also small microabscesses.

Fig. 1. Changes in the aorta of a monkey with pyridoxine deficiency. Animal No. 146. Colloidal Iron. ×80. Note elevation of the intima with accumulation of mucopolysaccharide.

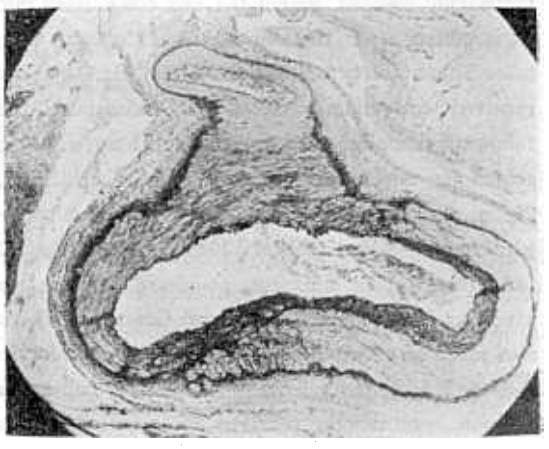

Fig. 2. Changes in muscular artery of a monkey deficient in pyridoxine. Animal No. 146. Aldehyde-Fuchsin. ×80. Note degeneration and fragmentation of elastic fibers in the intimal area.

Animal No. 188 showed slight intimal swelling of the muscular arteries and fatty changes in the liver. Animal No. 189 showed slight intimal swelling and thickening of the aorta, but was otherwise not remarkable. Slight fatty change in the liver was observed.

The group deficient in essential fatty acids: Animals No. 150, No. 152 and No. 154. This group of animals also showed sclerotic changes similar to those seen in the first group, but the distribution and the degree of change were somewhat different. The sclerotic changes appeared less severe than the first group, and there was no demonstrable fatty changes in the liver. The arterial alterations were mainly observed in the smaller arteries (Figures 3, 4).

Animal No. 150 showed slight intimal swelling and thickening of the renal arterioles. The coronary artery also showed a minimal degree of intimal

proliferation. The liver was not remarkable. The lung contained small granulomas probably due to parasitic infestation.

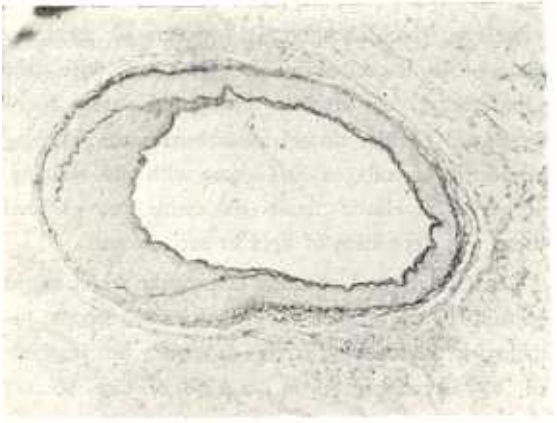

Fig. 3. Changes in the coronary artery of animal with essential fatty acids deficiency. Animal No. 150. Aldehyde-Fuchsin. ×80. Proliferation and hypertrophy of intima with fibrosis and duplication of elastic fibers is evident.

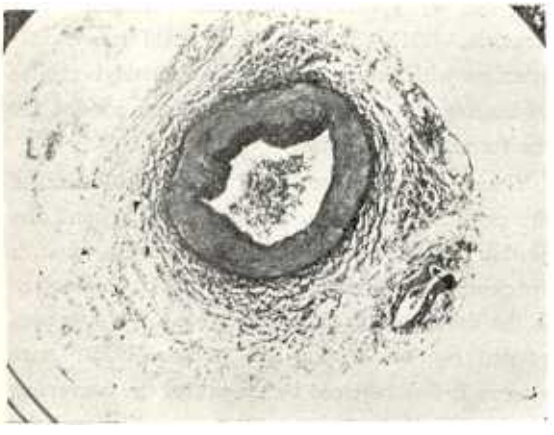

Fig. 4. Changes in muscular artery of a monkey with essential fatty acids deficiency. Aldehyde-Fuchsin. ×40. Note Changes in the intima.

Animal No. 152 showed sclerotic changes in the renal arterioles. The liver showed no fatty infiltration or metamorphosis, but contained microabscesses of unknown origin.

Animal No. 154 also showed slight sclerotic cha nges of the smaller arteries.

The group deficient in pyridoxine and essential fatty acids: Animals No. 160, No. 162, No. 182, No. 183, No. 184.

The sclerotic alterations of arteries seen in this group were definitely more marked and more severe than in the first two gronps of animals. The changes in the liver also were more marked and were characterized by marked fatty changes, and sometimes leading to cirrhosis.

Animal No. 160 showed slight thickening and proliferation of the intima of the aorta and renal arterioles but other arteries showed a remarkably high degree of deposition of sclerotic plaques. The liver showed marked fatty changes plus evidence of nodular cirrhosis. Focal necrosis of the liver parenchyma was also observed. The lung showed slight

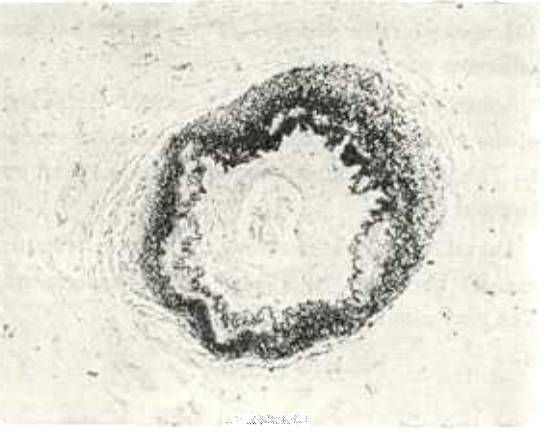

Fig. 5. Changes in muscular artery of a monkey with deficiency of both pyridoxine and essential fatty acids. Animal No. 160. Aldehyde-Fuchsin. ×60. Note changes in intima.

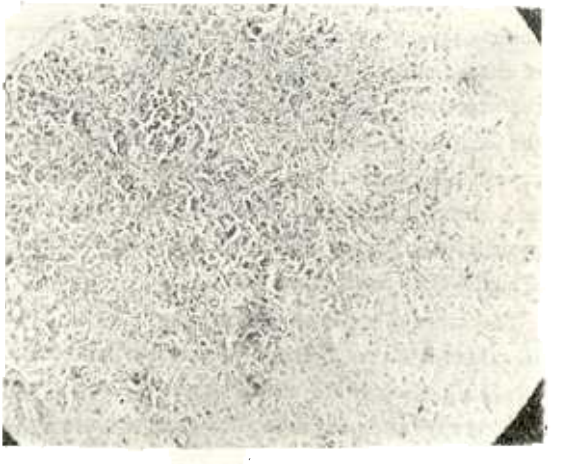

Fig. 6. Changes in liver of animal with deficiency of both pyridoxine and essential fatty acids. Hematoxylin and Eosin. ×60. Animal No. 162. Note cirrhotic changes.

hyperemia and focal granulomas, which were probably due to infestation by parasites.

Animal No. 162 showed histological changes more or less similar to those in Animal No. 160. The changes in the liver were also similar. Calcification of the adrenal medulla was seen in this case.

Animal No. 181 showed a moderate degree of sclerotic change in the renal arterioles. Muscular arteries chiefly showd prominent intimal proliferation. The changes in the liver were rather marked. The pancreas showed focal fibrosis. The lungs showed histological changes of bronchopneumonia, but the vessels showed no remarkable changes. Animal No. 182 showed fatty changes of the liver, but was otherwise not remarkable.

Animal No. 183 showed slight intimal thickening of the aorta, but the coconary artery was not unusual. The liver exhibited only fatty changes but no cirrhotic findings.

Animal No. 184 was remarkable in that the sclerotic changes developed quite early in the experiment. The liver also showed fatty changes.

DISCUSSION

The data obtained through this investigation tend to agree with the results reported by previous investigators who worked on similar subjects. (Rinehart and Greenberg, 1949; Mushett and Emerson, 1956) Although sclerotic vascular changes could be observed in all the experimental animals, the degree of change appeared to be related to the duration of the experiment. From an analysis of the severity of the lesions and the duration of such changes, these appear to be most marked in the combined pyridoxine and essential fatty acids deficiency, less marked in pyridoxine deficiency alone, and the least marked in essential fatty acids deficiency.

The order of severity appears to correlate with the changes in the liver also. Prominent fatty metamorphosis of the liver was observed in the first and third group, where as the second group did not show any appreciable changes in the liver. The combined deficiency led to the development of cirrhotic changes in the liver.

The appearance of the sclerotic changes in the aorta and arteries is certainly similar to that observed in the human counterpart. Various histochemical studies proved that the initial and the main alterations are brought about in the ground subsatance of vessels in which an abnormal amount of mucinous material is deposited. This coincided with the proliferation of intimal cells, and later there developed fibrillar material, some with the staining properties of collagen and some with the staining properties of elastic tissue. Fat stains proved that there was deposition of lipid in such lesions.

The fundamental chemical mechanism which might explain the occurrence of gross or microscopic pathology in tissues in pyridoxine deficiency was suggested by Greenberg et al. in 1958. They pointed out that the development of arteriosclerotic lesions was correlated with the formation of phosphatidly choline (lecithins), and that in pyridoxine deficiency there might be a block in the decarboxylation of serine to form ethanolamine, a reaction presumably catalyzed by pyridoxal phosphate (Stetten, 1941; Arnstein, 1951). Such a block could conceivably interfere with the synthesis of phosphatidyl choline by limiting the supply of ethanolamine needed for the formation of choline.

Vitamin B-6 was also linked to the metabolism of the essential fatty acids. Schroeder (1955) and also Sinclair (1956) postulated the possibility that a deficiency of essential fatty acids might be involved in the development of atherosclerosis. As has been pointed out by Witten and Holman (1952), since vitamin B-6 is required by the rat for the conversion of linoleic and linolenic acids to the higher polyunsaturated fatty acids, namely, arachidonic, pentaenoic, and hexaenoic acids, it is conceivable that vitamin B-6 deficiency might result in interference with the metabolism of the essential fatty acids. (Greenberg et al., 1958)

In view of the above results, attention is called to the essential role of vitamin B-6 in the metabolism particularly of proteins and its possible relation to the pathogenesis of human arteriosclerosis. The importance of the essential fatty acids in this respect cannot be discounted, either. Deficiency of these two factors in combination appears certainly to be

related to the enhancement of arteriosclerosis in monkeys.

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