Technical Papers

Abnormal Lipid in Coronary Arteries and Aortic Sclerosis in Young Rats Fed a Choline-Deficient Diet*

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Lipid deposits in walls of coronary arteries and aortas and grossly evident aortic sclerosis in young rats have been observed after short periods on a low-choline, high-fat diet. The lesions are of special interest because they developed rapidly in intact animals fed diets that did not contain supplements of cholesterol. These diets contained high levels of protein (30 percent) and fat (35 percent) and were relatively low in methionine (520 mg/100 g of food). We have previously reported vascular lesions in older rats maintained for longer periods on choline-deficient diets that contained lower levels of both fat and protein (*1*). Alterations in the vessels of the young animals are regarded as the acute form of the chronic lesions encountered in older rats.

One hundred thirty-seven young male rats (120 g) of the Wistar strain were used. Thirty-six of these were fed the basal diet (*2*) supplemented with 0.85 percent choline chloride. These controls were offered and, over the experimental period of 4 wk, consumed the same amount of food as did the 101 experimental animals of the same age and weight that ingested the basal, unsupplemented diet *ad libitum*. Control animals and survivors in the experimental group were sacrificed under ether anesthesia at the end of the 4-wk period. Half of the animals on the low-choline diet succumbed to acute hemorrhagic renal lesions before the end of this period.

Gross and microscopic examinations revealed that abnormalities of the cardiovascular system were absent in all 36 choline-supplemented controls but had developed in 22 of the choline-deficient rats (22 percent). In half of the latter, the sclerotic aortic changes were grossly evident throughout the thoracic aorta. In one instance, this had developed in a rat that died of acute renal injury after only 17 days of choline deficiency.

Microscopically, the aortic lesions consisted of intimal and medial deposits of lipid, frequently associated with pathological amounts of calcium salts in the media. Coronary arteries contained intimal and medial deposits of stainable fat (Figs. 1, 2), but calcification has not been observed in these arteries. In some cases, intimal hyperplasia was associated with abnormal intimal lipid (coronary arterial lipoidosis). In every instance in which fat was demonstrable in lining endothelial cells, the latter were swollen. Lipid deposits appear to constitute the initial stage in the pathogenesis of the lesions of both coronary arteries and aortas.

In a subsequent experiment (*3*), 40 male rats weighing on the average 100 g were used (85 to 125 g). These animals were younger than those in the initial experiment. Twenty rats were fed the basal, choline-deficient diet *ad libitum*, and the remainder were offered and consumed the same amount of the choline-supplemented (0.85 percent) diet. Fifteen of the choline-deficient rats succumbed either to hemorrhagic renal lesions or to cardiac necrosis (*4*). Autopsy revealed, on either gross or microscopic examination, that 18 of the 20 choline-deficient rats had developed lesions of coronary arteries or aortas or both, whereas at the end of 4 wk, when all animals were sacrificed, the vessels of the choline-supplemented rats were found to be entirely normal.

Lehr and associates (*5*) have described the rapid production of similar vascular lesions in rats in which renal injury followed administration of sodium N-acetyl sulfathiazole. Their findings suggest that renal injury induced by the low-choline diet may be an important factor in the production of the lesions described here. But Lehr *et al.* did not report lipid deposits in the affected vessels, and it may be that this feature characterizes the changes induced by choline deficiency.

We have observed grossly evident aortic sclerosis in the absence of what appears to be a significant degree of renal damage, but this is the rare exception to their otherwise constant association. Nevertheless, the exceptions may indicate that vascular and renal changes are not necessarily etiologically dependent.

Wissler *et al.* (*6*) have reported that choline supplements actually favor the development of lipomatous and atheromatous lesions in coronary arteries of rats

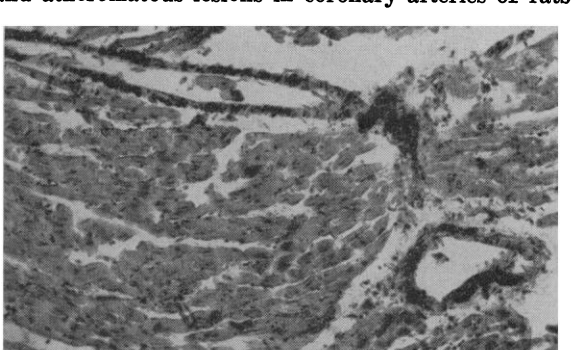

Fig. 1. Low-power (×50) photomicrograph of stem-branches of coronary arteries supplying the left ventricle of the heart of a rat that consumed the basal, choline-deficient diet for 17 days. Stainable fat (black) is deposited throughout most portions of the arterial walls. The surrounding cardiac muscle is relatively normal. Oil red O stain photographed through Wratten B and G filters.

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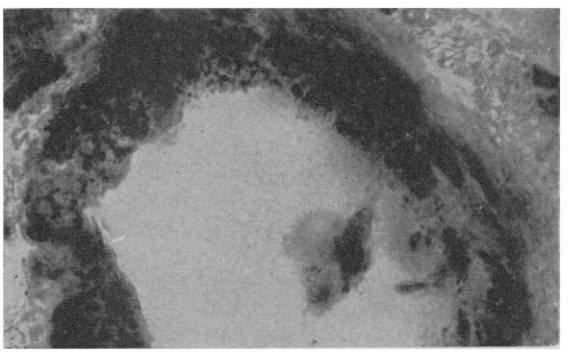

Fig. 2. High-power (×600) photomicrograph of similar preparation to that shown above. Masses of stainable fat (black) are deposited in all portions of the arterial wall. Lipid in intimal endothelial cells is in the form of fine droplets.

that received a high-fat, high-choline ration containing cholesterol. Lesions developed more rapidly in similar rats that received 1 percent NaCl in their drinking water and were given anti-rat-kidney serum, desoxycorticosterone acetate, and sesame oil injections. The arterial lesions were directly associated with the renal damage that these measures produce. Since choline exerted no protective effect in Wissler's experiments, it is possible that the lesions he has observed and those reported here are not closely related.

The lesions that McCormick and Holman (*7*) have described in dogs fed a high-fat diet following the production of a "standard" amount of renal damage may be more closely related to those that we have observed in choline-deficient rats. It would be interesting to know whether high levels of dietary choline would exert any protective influence on the vascular lesions seen in Holman's type of experiment.

Subsequent to our reports (*1*) concerning the production of vascular lesions in choline-deficient rats, Mann *et al.* (*8*) have produced atherosclerosis in *Cebus* monkeys by giving them large amounts of cholesterol in methionine-deficient diets. Methionine supplements prevented atherosclerosis in control animals. Mann has not specifically investigated the effects of choline deficiency on the vessels of monkeys, and it is possible that he is observing a manifestation of the lipotropic action of methionine in his experiments.

Earlier reports from our laboratory concerning the role of choline in the development of cholesterol-induced atheroma were limited to investigations in rabbits in which choline was added to a basal diet that already contained appreciable amounts of lipotropic factors. The effects of diets very low in lipotropic factors were not studied in that species (*9*).

We believe that the results of these studies using the rat indicate that an adequate intake of choline favors the maintenance of not only a healthy liver and kidney but also a normal cardiovascular system in young rats. Recent *in vitro* studies by Artom (*10*) have shown that the rate of fatty-acid oxidation by slices of livers, kidneys, and hearts of choline-deficient rats is enhanced if the animals are given choline just prior to the time of sacrifice. Morphologic lesions in all these tissues have been found to begin with stainable deposits of fat which precede the irreversible changes associated with cirrhosis, renal damage, and cardiac necrosis.

If it should prove that the vascular lesions reported here are primarily due to a relative lack of lipotropic substances in the diet, another of the factors necessary to maintain the healthy state of the walls of blood vessels will have been recognized. It will obviously be necessary to confirm these findings on other species of laboratory animals before their possible clinical significance is considered.

We suggest that the term *lipotropic* may now be extended to include the prevention of the accumulation of abnormal (stainable) fat not only in the liver and kidneys but also in the heart and blood vessels of animals that exhibit these lesions when choline and its precursors are withdrawn from the diet.

References and Notes

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Retardation of Maturation in Magnolia Flowers by Maleic Hydrazide

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It has been shown that MH (maleic hydrazide) inhibits or retards several processes in plants. Cell division is inhibited in the root tips of *Vicia faba* (*1*) and onion (*2*); respiration is retarded in onions through partial inactivation or inhibition of one or more of the dehydrogenases (*3*); softening of apples is retarded and respiration is reduced, as measured by the quantity of CO_2 given off (*4*); and in some cases MH appears to act as an antiauxin (*5*). Evidence is presented in this paper to show that MH retards the aging processes in the flowers of *Magnolia grandiflora* L., the ability of MH to inhibit these processes being correlated with the quantity of starch in the perianth segments at the time of application.