Vascular Disease Associated with Choline Deficiency in the Rat

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ALTHOUGH fatty liver[1] and hemorrhagic renal degeneration[2] as a consequence of dietary choline deficiency in the rat have been known for many years, it was not until 1952 that Hartroft, Best and their colleagues[3] described pathologic changes in the major arterial trunks (aortas and carotid arteries) and in the coronary arteries of rats maintained up to 216 days on diets low in choline. Somewhat later these studies were extended in our laboratory to the investigation of vascular changes in acute severe choline deficiency.[4] Following this, further work was undertaken to elucidate the etiology, pathogenesis, and biochemistry of the cardiovascular lesions in choline deficiency.[5]

A thorough investigation of this problem seemed to be strongly indicated in view of the great interest in the role of lipotropic factors in nutritional disorders as well as their possible significance in the vast complex of cardiovascular pathology.

HISTOPATHOLOGY

Young choline-deficient rats may develop a number of cardiovascular lesions after three weeks in acute, or after approximately five months in long term, experiments.

A focal cardionecrosis is found preceded by the appearance of stainable lipid within the muscle fibers (Fig. 1). Polymorphonuclear leucocytes and later lymphocytes appear at the site of lipid-laden muscle fibers. Interstitial edema is frequently pronounced. Sometimes death of the animals ensues before this interstitial myocarditis can further develop into frank focal necrotic areas. If the animal survives, the inflammatory and necrotic debris is absorbed and removed, leaving focal fibrotic scarred tissue in the myocardium. No definite topographic relation between the coronary tree and these focal necrotic areas has as yet been discerned.

Stainable lipid mainly in the media of coronary arteries is noted (Fig. 2). Intra- and extracellular lipid also may be observed in the intima and the adventitia but the location of main accumulation is the media. Sometimes this stage of lipid appearance is followed by signs of necrosis in the components of the media.

The same picture as in the coronaries is observed in the aorta of choline-deficient rats except that medial necrosis is much more pronounced and much more common. This medial necrosis is then followed by calcification and the aorta is frequently totally rigid, hardened, and widened with the appearance of a bamboo stick (Fig. 3). The intima of the aorta is but rarely involved and shows on occasion only a "hyaline cap" over areas that are necrotic and calcified in the underlying media. This hyaline cap is apparently a proliferative connective tissue response to the injury of the medial structures beneath.

The elastic elements of the aorta are shriveled and broken up. The whole media looks microscopically like a condensed calcified bar. On occasion this bar-like tissue breaks, leading to a picture that has been termed "collar-button fracture" by Hartroft. The whole syndrome may therefore most favorably be com-

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pared with Mönckeberg's sclerosis in human arteries (Fig. 4). There is no similarity to the process of atherosclerosis as encountered in man.

ETIOLOGY AND PATHOGENESIS

The etiology and pathogenesis of those lesions may be of little *practical* significance but it is a very important theoretic matter. It is obvious that a lack of choline initiates these lesions as they are all prevented by the addition of sufficient choline to hypolipotropic diets. The mechanisms by which these lesions are induced in choline-deficient rats are still obscure despite considerable work and effort put into this problem. The main point is whether these pathologic changes are due primarily to lack of choline or whether they are secondary to renal cortical necrosis that is nearly always present in choline-deficient animals suffering from cardiovascular disease. Bilateral renal hemorrhagic cortical necrosis is induced by severe choline deficiency and it is conceivable that the ensuing pre-uremia or uremia is responsible for the tissue break-down in the vessels. We favor this view of the nature of cardiovascular disease, i.e., as being secondary to renal damage in choline-deficient rats. Others, however, feel that lipotropic factors are primarily involved in the integrity of the cardiac tissues of the rat. Hartroft favors the opinion that choline is the essential dietary component necessary for the health of the cardiac muscle while vascular changes in the aorta and coronaries may perhaps be due to renal injury. He therefore thinks that cardiac necrosis on the one hand and vascular changes on the other, are due to different etiologic factors. This concept has some experimental support and seems to be very attractive. Kleinerman,[6] however, supports the concept that it is methionine which is the component essential for the prevention of

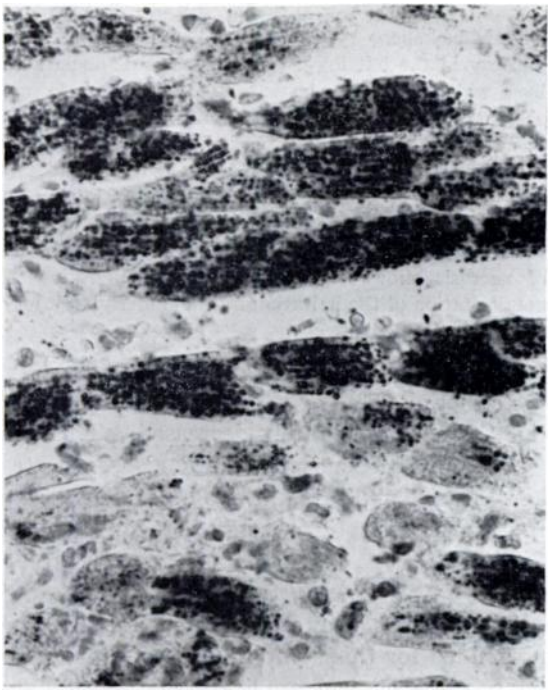

Fig. 1

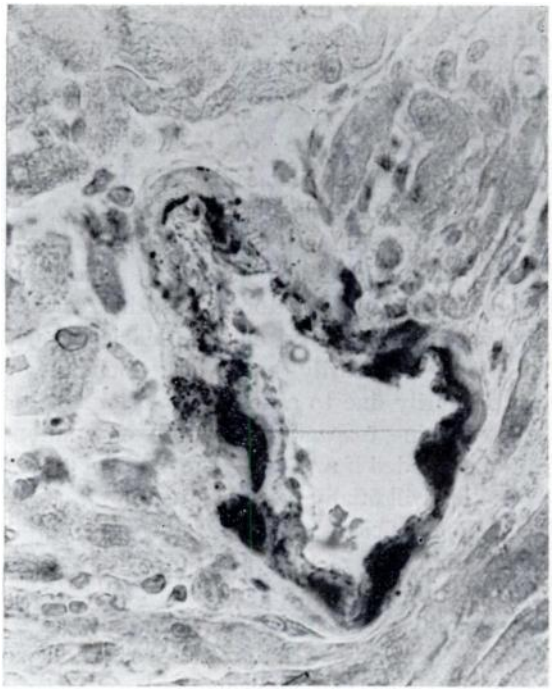

Fig. 2

Fig. 1. (High power, frozen section, Oil Red-O stain.) Fat appears as black droplets throughout the cytoarchitecture of choline-deficient myocardial fibres. The lipid accumulation within the muscle fibres is followed by edema and the appearance of polymorphonuclear leucocytes. Later on necrosis and lysis of the muscle fibres ensues. Fig. 2. (High power, frozen section, Oil Red-O stain.) Fat appears as black masses throughout the entire vessel wall in this coronary artery of a choline-deficient rat. Note, however, that the site of main lipid accumulation is the media.

Fig. 3. At autopsy the bamboo stick appearance of the aorta in a chronically choline-deficient rat is very striking. Note that the aorta is widened and that calcified rings characterize this state of aortic sclerosis.

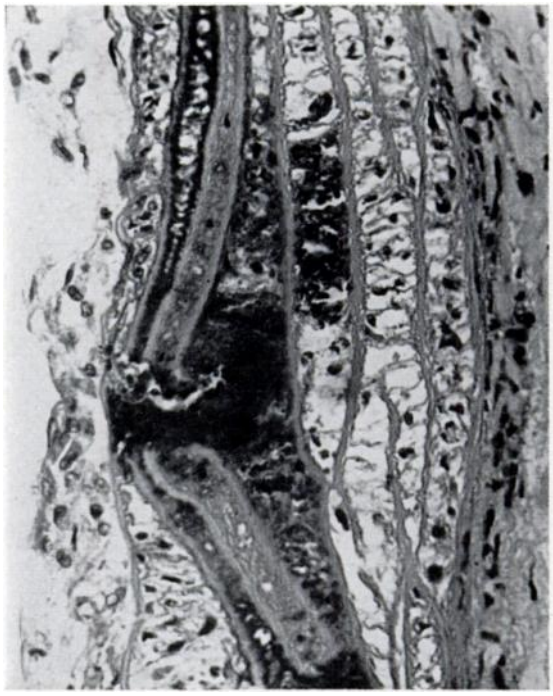

Fig. 4. (High power, paraffin section, PAS stain.) The Mönckeberg type of medial sclerosis, seen at autopsy in the left lower photograph is demonstrated by a widening of the elastic laminae of the aorta and accumulation of cellular debris and connective tissue disintegration. The inner part of the aorta is rigidly calcified into a bar that is broken. At the site of this fracture strongly PAS-positive material is illustrated by the darkness of the area surrounding both ends of the fractured bar. The epithelium overlying this site of necrosis shows signs of cellular proliferation as manifestation of the irritation by the underlying injury of the aortic media.

break-down of cardiovascular tissues in the rat. Since methionine is one of the biologic precursors of choline it is very difficult to exclude *some* methionine deficiency in a diet that is low in lipotropic factors. Whether cardiac and vascular lesions in choline deficiency are, therefore, secondarily due to lack of choline or are primarily due to a deficiency of choline or of methionine, respectively, can, in our opinion, only be decided if and when the mode of action of choline will finally be elucidated.

Choline-deficient animals always exhibit blood lipid values that are lower than their choline-supplemented controls. This holds true whether the blood lipids are expressed physicochemically as lipoproteins or biochemically as cholesterol, phospholipids or neutral fat.[7,8,9] In contrast to the low blood values are the levels of liver lipids in choline-deficient animals which, of course, are always elevated as compared with their choline-supplemented controls. In atherosclerotic humans and in experimental animals the blood lipid values are either elevated or within the normal range, but they are only rarely decreased. This difference in biochemical pattern also separates cardiovascular disease in choline-deficient rats from atherosclerotic processes in man.

CLINICAL APPLICATIONS

The interest of an audience such as the one assembled for this symposium is often focused on possible clinical applications. Both lipotropic phenomena and atherosclerosis command great attention these days. We feel obliged, however, to come to the conclusion that cardiovascular disease as observed in choline-deficient rats has no direct clinical bearing on the vascular lesions which are en-

countered most frequently in North America. The reasons are as follows:

(1) Pathologically, the lesions resemble Mönckeberg's sclerosis and not those of atherosclerosis. The sequelae of Mönckeberg's sclerosis are not nearly as deleterious to health as those of atherosclerosis.

(2) The biochemical lipid patterns in choline deficiency and in atherosclerosis are altered in opposite directions.

(3) A relationship between choline deficiency and human Mönckeberg's disease is at present not established because patients having Mönckeberg's vascular sclerosis do not seem to suffer from dietary lack of choline here in North America. Whether there is an intrinsic defect of choline metabolism in those patients remains to be investigated.

(4) A clinical application of the findings in choline-deficient rats to areas where malnutrition and kwashiorkor are common is perhaps possible. But here again the complex problem of protein malnutrition is hard to separate from an inadequate supply of choline in the diet. We have pointed out before that a choline-deficient diet is frequently border-line deficient in methionine as well, and therefore, choline and protein deficiencies are not easy to differentiate.

(5) So far, no species other than the rat has been observed to develop this type of vascular disorder in choline deficiency. This would lead one to assume that there is a specific reason for this susceptibility of the rat which does not occur in other species.

To make clinical applications of an experimental finding requires that several species including man should exhibit similar, or at least comparable results. As the vascular lesions in choline-deficient rats seem to be confined to this one species, it seems reasonable to be cautious about making any extensive clinical comparisons or applications.

Recently Williams[10] has described cardiac necrosis in choline-deficient mice. But here, too, as Dr. Williams points out,[11] protein deficiency may be the etiologic factor because even choline-supplemented controls may exhibit cardiac necrosis on those protein-poor diets.

THEORETICAL IMPLICATIONS

Although we feel that we cannot subscribe to a direct clinical application of the findings in choline deficiency we still believe that this type of work is of great importance. Choline is certainly of significance in the metabolism of fat in the liver and its release into the blood. The whole problem of lipid transport is currently being reinvestigated in many laboratories and choline seems to play some role in these complicated processes. A severe lack of dietary choline leads in rats to a breakdown of cardiovascular tissues and it would be of great importance to know by what mechanisms the integrity of the cardiovascular tissue structure is maintained.

While all agree that choline is a dietary essential for man as well as for animals it appears that normal mixed diets supply adequate amounts of choline or its precursors. The therapeutic use of *extra* dietary choline does not seem to be warranted on physiologic grounds. It is obvious, however, that a broad research program on the mode of action of choline will continue to make important contributions in many scientific fields as it has in the past.

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DISCUSSION

Dr. Gerald P. Rodnan (University of Pittsburgh, Pittsburgh, Pa.): So impressive have been the findings shown to us by Dr. Wilgram that for this reason alone I would be tempted to disagree with him about the possible clinical significance of these vascular lesions. To begin with, they are strikingly similar to those of so-called Mönckeberg senile sclerosis of man. Mönckeberg sclerosis is most apparent in the medium sized arteries, in such muscular arteries as the radial and femoral. It has been pointed out that the rat's aorta bears a closer resemblance to our medium sized arteries than to our aorta because, like the medium sized arteries, the rat aorta does not have vascularization of the media. The vasa vasorum penetrate no further than the outermost part of the adventitia.

It is true that in clinical material we do not observe the accumulation of lipids shown so beautifully in the sudan stains of Dr. Wilgram's material. Of course, we are dealing in the case of the human with a disease change which has evolved over a period of decades. We know very little about the earliest lesion of Mönckeberg's sclerosis. There is a degeneration of myoelastic fibers, but how this comes about we are quite unsure.

As far as the pathogenesis of these experimental lesions is concerned, a number of suggestions have been made. In the first communication on the subject, it was conjectured that the medial lesion of the aorta was secondary to damage of the intima. As a matter of fact, the title of the original paper was to the effect that the authors had produced atheromatous lesions in the intima of the vessel (*Proc. Soc. Exper. Biol. & Med.* 81: 384, 1952).

I agree with Dr. Wilgram that the intimal lesions are rather less striking, in the aorta certainly, than are the medial lesions. This is not hard to understand when one appreciates the fact that the rat aorta has a very narrow intima indeed, consisting only of a single layer of endothelial cells.

With regard to renal involvement as a basis for the cardiac and aortic findings, the evidence appears far from complete. Certain lesions have been seen in the myocardium of experimental animals and with renal insufficiency the so-called myocytolysis described recently by Schlesinger (*Am. J. Path.* 31: 443, 1955). These lesions, however, are not characteristically necrotic in character. There is none of the accumulation of inflammatory cells which we have seen in the slides of Dr. Wilgram's material. I would rather subscribe to the idea that these changes may be a result of choline deficiency itself. I wonder whether factors which protect in experimental choline deficiency, such as betaine and vitamin B_{12}, would also protect against these myocardial lesions, and whether such agents as cystine and choline antagonists which can produce fatty changes in the liver might enhance these lesions.

As far as aortic disease in general is concerned, there is one other lesion in the media of the rat aorta which is of great interest. A form of medial degeneration, frequently associated with aneurysm, has been produced experimentally in the rat by the administration of beta-aminopropionitrile. Given to young animals there results a deficiency of intact elastic laminae, fibroblastic proliferation, and in later stages a decrease in periodic acid-Schiff-positive material (*Arch. Path.* 64: 434, 1957). At times there is also involvement of the cardiac valves. I wonder if Dr. Wilgram has noted any alterations in the valves in his experimental animals.

Dr. Hartroft: I would make a plea that the term cardiovascular not be used too readily, lumping all these lesions together. I know we frequently say "cardiovascular," but here, at any rate for a while, let us keep the cardiac lesion separate from the vascular lesion and not speak about the aortic changes and cardiac necrosis as being the same thing until we learn more about them.

I think the evidence is pretty clear that cardiac lipoidosis and cardiac necrosis are not secondary to renal lesion because the original work which stimulated this work of Dr. Wilgram's and mine was that of Stetten, *et al.* (*J. Nutrition* 29: 171, 1945) who showed that feeding young weanling rats a choline-deficient diet containing 40 per cent of ethyl laurate killed them in three or four days. They died of myocardial necrosis. Stetten, *et al.* did not study the early stage of the precursor of necrosis, the lipoidosis. We were able to confirm them and study this early stage of cardiac lipoidosis. Dr. Stetten's rats did not develop renal lesions and Dr. Wilgram and I did not observe renal lesions in our rats either (*Brit. M. J.* 2: 1, 1954).

Secondly, I think we have a counterpart for this cardiac lesion in human pathology. In certain types of alcoholics, a very flabby necrotic, fatty heart may be encountered, mostly in beer drinkers. These lesions develop in acute alcoholics who have fatty livers. Perhaps the fatty necrotic heart in the rat is a morphologic counterpart of that lesion.

The concept of whether a deficiency of methionine or of choline is the important factor in the production of the vascular lesion is something which will be resolved only by further experimentation.

Dr. Lathem: Do these animals get hypertension?

Dr. Wilgram (closing remarks): In answer to Dr. Rodnan's comments I would like to say that when I used the term Mönckeberg's disease I used it with some reservations. I am quite aware that different interpretations may be ascribed to those different lesions. It is a matter of preference and one man likes to describe those experimentally produced lesions as cystic medial (Erdheim) necrosis and others prefer to call them Mönckeberg's medical sclerosis. Again I would like to point out that I am making these comparisons in terms of the anatomy of the rat realizing that there are differences between the anatomy of the rat and the anatomy of man. Certain anatomic lesions seen in humans just cannot be found in other species! Certain features however may be seen in both species. On the basis of those common features I have chosen, perhaps frivolously, to compare those lesions with the Mönckeberg lesion in man.

Dr. Rodnan: I agree with you that there is a striking resemblance pathologically between those lesions and Mönckeberg's sclerosis in man. The circling of the vessel with calcium and many of the other findings are quite identical with what is found in the human material.

Dr. Wilgram: Coming to the second point, the occurrence of necrosis in the myocardium, I should like to say that myocardial necrosis as a consequence of kidney damage may be observed under a variety of conditions. Time does not permit my going into detail, but I could give you five different references to kidney lesions produced by different experimental means with ensuing cardiac lesions, not identical, but similar to the ones observed here.

The third question concerned involvement of cardiac valves. I have as yet, not observed lesions in the valves.

The effect of ethyl laurate upon the myocardium of the rat mentioned by Dr. Hartroft is a problem which should be reinvestigated. It is true that in those early days kidney damage was not always observed. However, Dr. Hartroft, ethyl laurate is a highly toxic substance. One out of five of our controls (*Brit. M. J.* 21: 1, 1954) showed cardiac lesions, i.e., the triglyceride of lauric acid produced some cardiac necrosis in choline-supplemented controls. This is why at the present time I am inclined to pay less attention to those early experiments.

However, I would like to re-emphasize the fact that the whole question of pathogenesis and etiology is wide open, and I cannot insist that my view is right. I just mention it for the sake of discussion. Maybe I am wrong. The future will show.

Concerning hypertension, I believe Dr. Hartroft found some years ago that in the active stage of the development of hemorrhagic kidneys hypertension develops, but if they stay on a choline-deficient diet the hypertension disappears.

Dr. Hartroft: The rats do not develop hypertension during the acute stage, only during the recovery stage.