Lipoproteins in Choline Deficiency

By GEORGE F. WILGRAM, M.A., M.D., LENA A. LEWIS, PH.D. AND J. BLUMENSTEIN, B.A.

Choline deficiency leads to lipomatous infiltration of the coronary arteries and to aortic sclerosis of the Moenckeberg type in young rats. In this form of experimental cardiovascular disease the total serum cholesterol and the triglyceride-bearing lipoproteins are decreased. The beta-lipoprotein classes are essentially within normal limits. The significance of these findings in particular and of serum lipid values in general as relating to different types of experimental cardiovascular disease is discussed.

RECENT work in our laboratory has shown that the serum lipid fractions may be considerably changed in choline-deficient rats. (Also personal communication, Patterson.) It was felt, therefore, that lipoprotein studies might cast more light upon the problem of fat transport in the plasma of choline-deficient animals. Because choline deficiency leads to cardiovascular lesions in rats, it seemed important to find out whether or not there was any correlation between biochemical changes in the blood and the occurrence of cardiovascular lesions. This is particularly true, because many workers have contended that in humans, hyperlipemia, hyperlipoproteinemia of the beta-classes (S 20-40) and hypercholesterolemia are concerned in the pathogenesis of human atherosclerosis.

Our studies revealed that all the lipid-bearing protein fractions are decreased when high-fat choline-deficient diets are fed to rats over a period of four weeks. The significance of this finding in relation to the production of cardiovascular disease by means of choline deficiency shall be discussed.

METHODS

One hundred male and 10 female Wistar rats, weighing around 135 Gm. at the beginning of the experiment, were used. Fifty-five male rats were offered a high fat, choline-deficient diet while 35 animals served as choline-supplemented controls. Ten male rats were put on a completely different synthetic low fat, high protein choline-supplemented diet to be compared with the effect of our high fat choline-supplemented standard diet (table 1). Ten female animals were used as choline-deficient controls to be compared with the choline-deficient males. No choline-supplemented female group was started, but this will be done in the near future. All animals were fed ad libitum. They were starved, however, on the day before sacrifice. They received food ad libitum for two hours, early in the morning of the day of sacrifice; five hours later the animals were killed (20 per day); the conditions of sacrifice were identical for all animals.

Table 1.—Composition of Diets

<table>
<thead>
<tr>
<th></th>
<th>WA-0</th>
<th>WA-1</th>
<th>WF-0</th>
<th>WF-1</th>
<th>WF-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut Meal*</td>
<td>20</td>
<td>20</td>
<td>28</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>Soy protein†</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Salt mixture‡</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sucoase-vitamin mixture§</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cellulour</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20</td>
<td>19.15</td>
<td>21</td>
<td>20.15</td>
<td>50.7</td>
</tr>
<tr>
<td>Lard</td>
<td>54</td>
<td>54</td>
<td>34</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>α-tocopherol acetate§</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Cod oil §</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>—</td>
<td>0.85</td>
<td>—</td>
<td>0.85</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Extracted with 50, 75 and 95 per cent ethanol.
† Water washed "alpha proteins" (Glidden Co.).
§ The fat-soluble vitamins are dissolved in corn oil so that one per cent of dietary corn oil will supply the desired amount of fat-soluble vitamins.
¶ Obtained from Ayerst, McKenna and Harrison Ltd., Montreal. Contains 200,000 I. U. vitamin A and 50,000 I. U. vitamin D per gram.
were kept constant on five successive days of killing. The animals were injected with 1 cc. of a 1 per cent sodium amyotal solution per 100 Gm. of body weight and exsanguinated by exposure and severance of both carotid arteries. This procedure seemed to secure a maximum and constant food intake on the day of sacrifice and there is good reason to believe that food absorption from the intestine was at approximately the same stage in each animal.

The serum lipoproteins were studied ultracentrifugally by Lewis and coworkers' modification of the Gofman technic.7 Cholesterol was determined by the method of Abel and associates.8

RESULTS

Of 55 male choline-deficient rats, 25 died from hemorrhagic kidney lesions which occurred as a consequence of lack of choline. The remaining 30 animals sustained a crisis of kidney damage and recuperated. They ate well and were gaining weight again; after being four weeks on the experimental diets, long enough to make them markedly choline-deficient, they were sacrificed. Female animals are much more resistant to the development of hemorrhagic kidneys in choline deficiency and no losses were incurred out of the 10 female animals which consumed the standard choline-deficient diet. There was no death among the choline-supplemented group in which all animals were perfectly healthy.

All choline-deficient animals had fatty livers4 (grade III-IV) upon autopsy. The kidneys of the male choline-deficient animals showed frequently slight chronic renal damage ("frosting"—Hartroft10), but the kidneys of the female choline-deficient animals were essentially free of lesions. The choline-supplemented controls showed no significant pathological changes. The results of the lipoprotein studies are listed in table 2. The data show that choline-deficient animals showed a marked decrease of the low density lipoprotein fractions (-S 70-400) which carry the main bulk of triglyceride.11 The alphalipoproteins of the -S 1-10 and -S 10-15 class are also lowered in choline-deficient male animals. These classes carry a high proportion of phospholipids.12, 13 The total cholesterol levels were decreased in choline-deficient animals as compared with the choline-supplemented controls.1 The beta lipoprotein classes (-S 20-40), however, showed no remarkable changes.

The ten rats on the high protein, low fat control diet which, too, was supplemented with choline, showed less low density lipoproteins than the choline-supplemented group on the high fat diet but still considerably more than all the choline-deficient animals. Remarkable is the high amount of alpha-lipoproteins in this control group on the high protein low fat diet. The results of serum protein studies carried out by paper electrophoresis showed in choline deficient animals only very slight variations from the choline-supplemented rats. This finding is consistent with other liver function tests performed in early choline-deficiency.14 The liver cells, although accumulating fat, are apparently not essentially disturbed in most of

TABLE 2 —

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sex</th>
<th>No. of Rats</th>
<th>Choline %</th>
<th>Total Cholesterol</th>
<th>Lipoproteins*</th>
<th>Total Protein Gm./100 ml Serum</th>
<th>Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>25</td>
<td>0.85</td>
<td>91.4</td>
<td>34.5</td>
<td>26.6</td>
<td>53.4, 16.5, 21.2, 8.9</td>
</tr>
<tr>
<td>WA-0</td>
<td>M</td>
<td>10</td>
<td>0.85</td>
<td>57.2</td>
<td>6.1</td>
<td>21.7</td>
<td>52.7, 17.2, 23.0, 7.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>10</td>
<td>0.85</td>
<td>70.0</td>
<td>33.0</td>
<td>25.4</td>
<td>50.3, 16.5, 19.1, 14.1</td>
</tr>
<tr>
<td>WF-0</td>
<td>M</td>
<td>11</td>
<td>0.85</td>
<td>63.4</td>
<td>7.0</td>
<td>24.6</td>
<td>48.8, 15.0, 23.2, 13.0</td>
</tr>
<tr>
<td>WF-0</td>
<td>F</td>
<td>10</td>
<td>0.3</td>
<td>74.0</td>
<td>16.3</td>
<td>26.5</td>
<td>173.5, 5.5</td>
</tr>
<tr>
<td>WF-4</td>
<td>M</td>
<td>10</td>
<td>0.3</td>
<td>74.0</td>
<td>16.3</td>
<td>26.5</td>
<td>173.5, 5.5</td>
</tr>
</tbody>
</table>

* Determined at d 1.21, using NaCl-KBr.
† Paper electrophoresis, barbiturate buffer pH 8.6, 0.05.
their metabolic functions in the early stages of choline deficiency.

**DISCUSSION**

Choline deficiency is one of the numerous procedures which may be used for the induction of cardiovascular disease in rats (cardiac necrosis, coronary lipidosis and aortic sclerosis). It is not known yet whether the occurrence of vascular lesions is primarily due to choline deficiency per se or is secondary to the renal damage (induced by choline deficiency). Irrespective of the etiology, however, it can be stated that these lesions occur with all lipid fractions (cholesterol, neutral fat, phospholipid) being decreased in the plasma. This proves the assumption that the cardiovascular system, even in the same species, may be damaged by a variety of mechanisms and that elevation of the lipids in the serum is not always a necessary prerequisite for the induction of arterial disease for all experimental procedures. In short, as Page 17 has pointed out, atherosclerosis is a multifaceted disease in which a variety of conditions lead to arterial disease of a more or less similar nature. The rat is a species rather resistant to the induction of lipomatous or atheromatous lesions in the coronaries and the aorta. Page and Brown 17 and Wissler and associates 18 produced fatty infiltration of blood vessels of the rat by severe hyperlipemia and hyperbetalipoproteinemia 17 or hypertension. 18 The elevation of serum lipids may or may not have some bearing on atherogenesis in humans and on many forms of experimental arteriosclerosis, but our results illustrate that cardiovascular changes may be produced experimentally without hypercholesteremia, hyperlipemia and hyper B lipoproteinemia.

It must be emphasized that pathogenesis and etiology of cardiovascular lesions in rats, caused by choline deficiency, are different from those of human atherosclerosis and from many other forms of experimental arteriosclerosis—e.g. the lesions in rats discussed above. 17, 18 Nevertheless, that fact remains that at least some forms of arterial disease may be produced with decreased lipid levels in the plasma. It is conceivable that primary tissue factors are more involved in choline-deficient cardiovascular disease than changes in the plasma biochemistry. In connection with kidney damage it is interesting to note that the lipoprotein changes observed in the nephrotic syndrome in the rat 20 are exactly opposite to those observed in choline-deficient animals. This observation too would indicate that pathological lesions in the kidney, even of seemingly similar character, are accompanied by different biochemical changes in the blood.

**SUMMARY**

The low density and alpha-lipoproteins are decreased in male rats fed a high fat, choline-deficient diet.

Consequently, their elevation would not seem to be a prerequisite to the production of cardiovascular disease in choline deficiency.

**ACKNOWLEDGMENT**

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**REFERENCES**


14 — AND BROWN, H. B.: Induced hypercholes-


On Clinical Experimentation

The student of disease should be certain that he is trying to learn about disease and not merely exercising his technical skill. One needs only to recall some of the absurdities and futilities of the iatro-mathematical and iatro-physical and iatro-chemical schools of the seventeenth century to realize the dangers inherent in this attitude of mind. Sanctorius is said to have spent forty years of his life in weighing himself three or four times a day.

Furthermore, there has grown up a certain sanctity about the word experimentation which seems to me to be unjustifiable. Experiments are of two kinds: first, the true experiment carried out to test a hypothesis; and second, the more or less random procedure undertaken to see what may happen. These latter experiments, made without hypothesis, can have only one purpose, and that is, to afford opportunity for observation. As Claude Bernard pointed out, such experiments are at times valuable since, in making the observations, hypotheses are suggested, and these can then be verified or disproved by true experimentation. But the student of medicine has little need for such groping for material. He is daily surrounded by phenomena which are stimulating beyond measure if he has eyes to see.

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