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THE CHOLESTEROL OF HYALINE ARTERIOLOSCLEROSIS *

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The more frequent occurrence of hyaline changes in the arterioles of the kidney in hypertensive persons than in those without hypertension has been generally accepted. The studies reported in this paper were inaugurated for the purpose of determining the nature of the hyaline material by histochemical means, and its relationships to atheromatosis of larger arteries and to hypertension.

Histochemical study of the hyaline material of renal arteriolosclerosis discloses both lipid and non-lipid content.[1] The lipid part absorbs sudanophilic dyes readily, such as Sudan IV.[2] The Schultz reaction for sterols gives the characteristic greenish reaction, indicating a cholesterol content of the hyaline regions of renal arteriolosclerosis.[1]

We have applied the Schultz modification of the Liebermann-Burchard test for cholesterol to the kidney in 47 cases of hyaline arteriolosclerosis and in 20 control cases. The test has been applied also in correlative histochemical studies to extrarenal arterioles, to arteries, and to other tissues of the body.

MATERIALS AND METHODS

The method of Schultz for cholesterol, based on the Liebermann-Burchard reaction, was applied as follows.[3] Frozen sections of formalin-fixed tissue, cut at approximately 15 μ, were placed in a mordant of 2.5 per cent aqueous solution of ferric alum for 3 days at room temperature. A section was rinsed in distilled water and blotted dry on a slide. It was then treated with the Schultz reagent, which consisted of equal parts of concentrated sulfuric acid and glacial acetic acid. The reagent was applied to the section by a glass rod, a coverslip

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was added, and microscopic study was made. The green color of a positive reaction appeared in a minute or so and lasted about an hour. The formation of air bubbles could be controlled somewhat by careful blotting of the tissue and by use of H_2SO_4 of at least 98 per cent purity. The strong acid altered the tissues but structures could be recognized by cutting down the light. No counterstain was applied. Since preparations were not permanent, photographs were taken for record.

Forty-seven cases with hyaline arterioles, and 20 control cases were tested with the Schultz reagent.

Selection of cases of renal arteriolosclerosis was made by examination of a single, routine, hematoxylin and eosin stained, paraffin section from each of many necropsies until 47 cases were located which exhibited hyaline arterioles. These were found in persons between 20 and 80 years of age. Accordingly, control cases devoid of hyaline arterioles in routine sections were selected from the same age period. Selection was made by an examiner unaware of other features of the case.

Correlative studies were made of many other organs and tissues.

Results

Of the 47 cases with hyaline arterioles in paraffin sections, Schultz-positive arterioles were identified in 43. Sudanophilic arterioles were demonstrated in 46 cases.

Of the 20 control cases with no hyaline arterioles in a routine hematoxylin and eosin section, Schultz-positive arterioles were demonstrated in 2, Schultz-negative arterioles in 14, while arterioles were not identified in similar preparations in 4. Sudanophilic arterioles were demonstrated in 4 of the 20 cases.

Correlative studies on one of the cases of arteriolosclerosis showed hyaline arterioles to be Schultz-positive in spleen, pancreas, liver, adrenal glands, and striated muscle. These arterioles were sudanophilic also. No hyaline arterioles were found in cardiac muscle, testes, skin, brain, and adipose tissue, and the Schultz test and Sudan staining also were negative in these arterioles.

The atheromas of arteries were consistently Schultz-positive (Fig. 6). These were demonstrated in aorta, common carotid, cerebral, splenic, and coronary arteries. The atheromas were sudanophilic but not hyaline.

Other tissues showed Schultz-positive lipid material in the adrenal cortex, in myelin of the nervous system, and in several other sites.

Concerning the relationship of hyaline arteriolosclerosis of the kid-

ney to hypertension, additional information was gathered. In 46 of the 47 cases the heart weighed more than 300 gm. In 16 of the 20 control cases the heart weighed more than 300 gm. The average of the 47 hearts was 497 gm., and of the 20 control hearts, 381 gm. Thus there was a tendency toward a greater degree of cardiac hypertrophy in the cases with hyaline arterioles in the kidney.

While hypertrophy of the heart in the absence of valvular disease is not considered by all to be an index of hypertension, many would agree that this increased weight of heart was suggestive evidence that hypertension had been present. It was impossible in many cases to say whether or not hypertension had been present during life, since many

TABLE I
Age of Cases Selected for Study of Cholesterol in Arterioles

Decade	47 cases	20 controls	Decade	47 cases	20 controls
Third	2	2	Sixth	6	
Fourth	6	4	Seventh	14	4
Fifth	10	4	Eighth	9	6

patients were in circulatory failure and had not been in the hospital long. However, 34 of the 47, or 72 per cent, had shown diastolic pressures over 100, while only 2 of the 20 controls, or 10 per cent, had shown diastolic pressures over 100. These data suggest a relationship between renal hyaline arteriosclerosis and hypertension.

Hyaline arteriolosclerosis did not appear to be merely a function of age. It occurred in the third and fourth decades and yet failed, at times, to occur in the eighth decade among the controls (Table I).

DISCUSSION

The Specificity of the Schultz Test

While Schultz[3] described his method for cholesterol in tissues in 1924, it has not been as widely used as it deserves. Knouff, Brown, and Schneider,[4] in 1941, used one adrenal gland of a guinea-pig for histochemical and the other for chemical examination and concluded that the Schultz histochemical test appeared adequate for distinguishing wide variations in the cholesterol content of the adrenal gland. It is usually stated that color reactions of this type are characteristic of steroids which possess some degree of unsaturation. Everett[5] wrote (1947): "The substances which give the characteristic color response in the Schultz test are evidently limited to the diols such as are formed from cholesterol by mild oxidation procedures." Cholesterol is the

characteristic sterol of higher animals and occurs in largest amounts in brain, adrenal glands, and egg yolk.

In our correlative histochemical studies with the Schultz reaction we found adrenal cortex and myelin of the central nervous system to give vivid color response. This corresponds with the chemical results. Most cells of the body, however, did not give a positive Schultz reaction, even though it is generally stated that all cells have cholesterol in them. This may indicate that the general statement is untrue, or that the test is not sensitive to minute amounts, or that the cholesterol is bound to other substances so as to prevent a positive test.

Among pathologic lesions, xanthomas, atheromas of larger arteries, and macrophages of cerebral infarcts gave positive Schultz staining and were sudanophilic also. Extrarenal hyaline arterioles, encountered in generalized arteriolosclerosis, were usually Schultz-positive and sudanophilic.

Sudanophilia and Cholesterol Test

The absorption of Sudan IV by fatty substances indicates only fatty or oily nature. As might be expected, therefore, several tissues were sudanophilic, but not Schultz-positive. This was true of adipose tissue, sebaceous glands, the lipid of fatty change of liver, lipochrome of heart muscle and of ganglion cells. Substances which were Schultz-positive were usually sudanophilic. This was true of adrenal cortex, atheromas, and hyaline arterioles. Myelin of the central nervous system, however, gave a strongly positive cholesterol test, but was not sudanophilic. With respect to the cholesterol of hyaline arterioles, sudanophilia appears to run closely parallel to the positive cholesterol test, and it is probable that generalizations concerning sudanophilia of the arterioles would likewise apply to a positive cholesterol test on them.

Wilens and Elster[2] (February, 1950) concluded that sudanophilic lipid deposition in the walls of renal arterioles occurs as commonly as it does in the intima of large arteries, and that its incidence is significantly increased in hypertension and in diabetes. Our studies add the information that the lipid substance of the hyaline arterioles gives a positive Schultz test for cholesterol, as do the atheromas of larger arteries. In a publication of May, 1950, Baker and Kent[1] presented evidence that the hyalin of arteriolosclerosis may have components other than lipid and other than cholesterol.

The Significance of the Cholesterol Deposits in Arterioles

Cholesterol deposits in arterioles may have the same significance as such deposits in larger arteries. It is possible that the lipid is imbibed

from the blood stream directly and that the increased incidence in hypertension and diabetes represents a driving of blood lipid into the wall. Such a mechanism would be even more reasonable if applied to the arterioles than if applied to the arteries because the thin arteriolar wall is in closer contact with the blood. On the other hand, it has not been shown clearly, as yet, that lipid change in vessel walls may not be due to local changes of a degenerative or regressive nature, perhaps like the fatty change of liver or heart muscle.

The change in the arterioles is like that of the arteries in that the lipids are apparently chiefly cholesterol or cholesterol esters in the two regions. In the arteriolar lipidosis, however, there is no tendency for the cholesterol to occur in crystals or in macrophages. Nor is there any apparent tendency for calcium to be deposited in conjunction with the lipid. This may be merely a matter of the smaller amount of material in the arteriolar wall, and the crystals and lipophages in the larger masses of atheromas may be secondarily derived from imbibed cholesterol. In one other respect there is dissimilarity, for the lipid-containing arteriole stains regularly like hyalin in hematoxylin and eosin sections while the atheromas do not exhibit this feature impressively.

Perhaps the bulk of evidence suggests that arteriolar lipidosis is the result of hypertension. Can we say definitely, however, that arteriolar lipidosis, by producing stenosis of arterioles, may not be a cause of hypertension?

Relation of Age, Sex, and Hypertension to Cholesterol Deposits in Arterioles

It is probable that the same conclusions can be drawn with respect to cholesterol staining as to sudanophilia of these arterioles. Wilens and Elster,[2] in a thorough study of the factors governing sudanophilia of arterioles, concluded that sudanophilia is relatively infrequent and slight in the very young and increases in incidence slowly but progressively with advancing age in non-hypertensive persons; that it is somewhat more common in hypertensive or diabetic women than in similar groups of men; and that there is no difference in sex incidence of renal arteriolar lipidosis in non-hypertensive persons or in whites and Negroes.

Further, these authors[2] found that the incidence of renal arteriolar lipidosis is significantly increased in all forms of hypertension except that associated with chronic glomerulonephritis; that it is significantly increased in diabetes; that it is not increased in renal diseases that are not associated with hypertension. They found, also, that the incidence

and severity of renal arteriolar lipidosis is increased in the presence of arteriolar sclerosis, although scanty lipid deposits are frequently found in arterioles that are otherwise unaltered.

Summary and Conclusions

Hyaline arterioles of the kidney were found to contain, in 43 of 47 cases, a lipid material which, on the basis of the Schultz modification of the Liebermann-Burchard test, was cholesterol.

Control studies on 20 cases indicated that in the absence of hyaline arterioles, in a single routine section of kidney from the necropsy, lipid in general and cholesterol in particular were less frequently demonstrated in the arteriolar walls.

Correlative studies showed the cholesterol test to be positive for hyaline arterioles of other organs, for atheromas of larger arteries, and for adrenal cortex and myelin. Adipose tissue and lipochrome failed to give a positive cholesterol test, although they were sudanophilic. Conversely, myelin gave a positive cholesterol test, though it was not sudanophilic. The positive cholesterol test in hyaline arterioles correlated well with the sudanophilia of these arterioles.

Since the hyalin of renal arteriolosclerosis contains cholesterol, like atheromas of larger arteries, a similarity of arteriolosclerosis to atheromatosis is indicated.

The hyaline arteriolosclerosis in hypertensive persons is accompanied by cholesterol deposition, on the basis of the Schultz test, more frequently than in non-hypertensive persons.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 81

FIG. 1. Hyaline arteriolosclerosis of kidney. Paraffin section. Hematoxylin and eosin stain. The hyaline, eosin-staining regions give a positive Schultz[3] test for cholesterol in appropriately treated frozen sections, more frequently in hypertensive than in non-hypertensive persons. × 103.

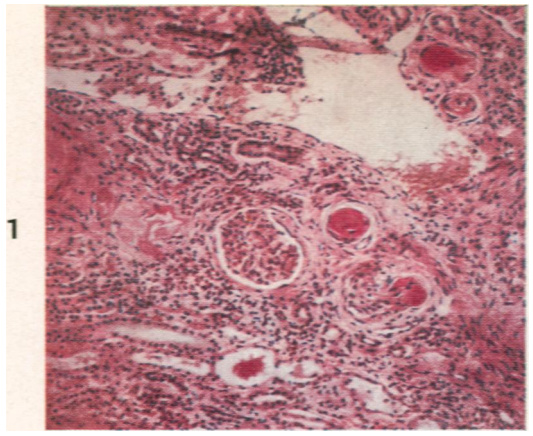
FIG. 2. Hyaline arteriolosclerosis of kidney. Frozen section. Sudan IV and hematoxylin stains. The sudanophilia indicates the fatty nature of the hyaline region, but is less specific chemically than the Schultz test. × 227.

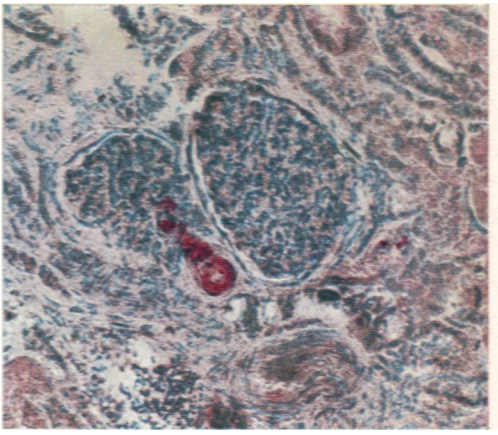
FIG. 3. Hyaline arteriolosclerosis of kidney. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. The green positive reaction indicates cholesterol in the arteriolar wall, on the basis of this reaction. × 94.

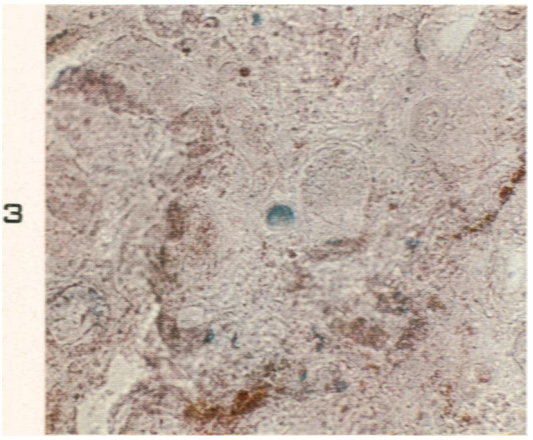
FIG. 4. Hyaline arteriolosclerosis of spleen. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. × 94.

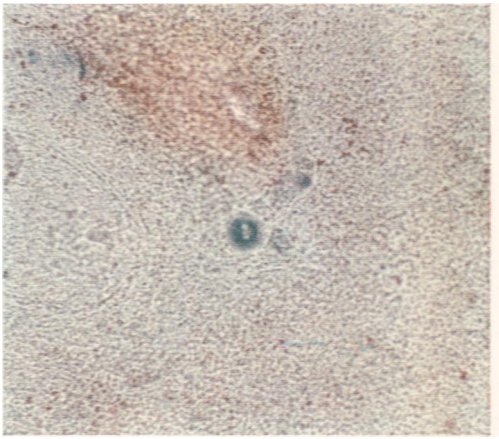
FIG. 5. Hyaline arteriolosclerosis of pancreas. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. × 97.

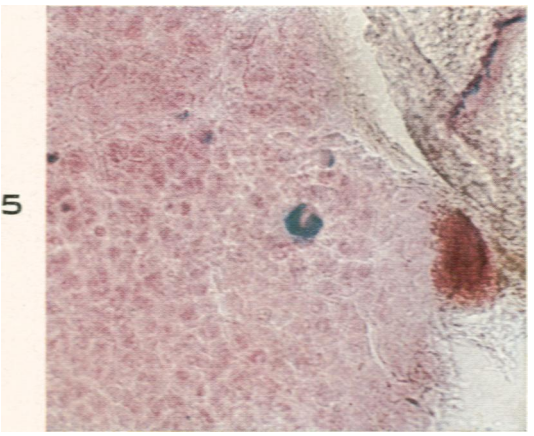
FIG. 6. Arteriosclerosis of aorta. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. The cholesterol test is positive in atheromata of arteries as well as in the hyaline regions of the arterioles. Chemical tests have shown the high cholesterol and cholesterol-ester content of atheromata of arteries. Presumably, chemical tests would show the same for arterioles, on the basis of the histochemical studies reported herewith. × 14.3.

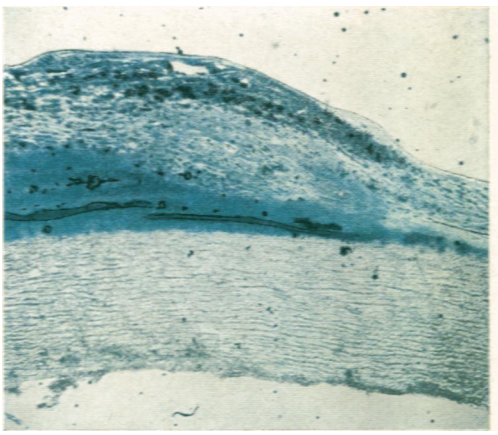












Baker and Selikoff Cholesterol of Hyaline Arteriolosclerosis