A CHARACTERIZATION OF HYALINE ARTERIOLAR SCLEROSIS BY HISTOCHEMICAL PROCEDURES *

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Arteriolar sclerosis and benign nephrosclerosis have been considered to be the integral morphologic features of benign hypertension, although the occurrence of similar changes in non-hypertensive subjects, particularly after the age of 50, has been emphasized.[1] In the latter circumstance the vascular sclerosis is less marked and less common than in hypertensive subjects. The position of arteriolar sclerosis in the general problem of hypertension has been stressed by Goldblatt[2] who has considered lack of knowledge of the nature and origin of arteriolar sclerosis as one of the outstanding deficiencies in the understanding of the pathogenesis of essential hypertension.

The correlation between cardiac enlargement and renal contraction depicted by Bright[3] was followed by the description of Johnson[4] of the thickening of small arteries in hypertension and the designation of arterio-capillary fibrosis by Gull and Sutton[5] in 1872. From that time until 1937 descriptions of the vascular lesion of hypertensive cardiovascular disease emphasized mainly thickening of the wall and narrowing of the lumen of arterioles. In 1937 the classical discussion of Moritz and Oldt[6] clarified certain outstanding morphologic features of arteriolar sclerosis. These authors recognized the involvement of various layers of the vessel wall and emphasized particularly three disturbances; namely, endothelial hyalinization, medial hypertrophy and degeneration, and endothelial hyperplasia. Referring to intimal hyalinization, they stated:

"This was the most common form of chronic arteriolar disease observed and consisted of a subendothelial accumulation of homogeneous, acidophilic material which appeared to represent an infiltration or expansion of the ground substance between smooth muscle of the media and the endothelium. . . . Although the principal mass accretion of hyalin was commonly inside of the internal elastic lamella, it appeared that the hyalin actually enveloped the elastic lamella which in many vessels lay approximately in the center of the hyaline mass. In such circumstances, elastic degeneration was invariable and was represented by swelling, disruption and dispersion of fibers with eventual complete disappearance."

The presence of connective tissue elements, as in endothelial hyperplasia[6] and medial fibrosis,[7] appears to be generally agreed upon, but

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the nature of the hyaline substance in the vessel wall remains obscure.

Recently this question has been reviewed and discussed by Duguid and Anderson[8] who, by conventional histologic examination, lent emphasis to the concept that the hyaline substance is derived from the circulating blood. This concept considers that the infiltrate accumulates next to the endothelium and is eventually covered over by the endothelium, while, at the same time, it displaces the normal constituents of the vessel wall, eventuating in a homogeneous replacement of the various layers, associated with thickening of the wall and narrowing of the lumen.

The obscurity of the origin of the hyaline substance is characterized by the treatment of this substance in textbooks. Bell[9] considered that the muscle of the arteriole is replaced by hyalin, but the lumen remains open, and Boyd[10] stated that the appearance suggests an accumulation or deposition of hyaline material which leads to narrowing and, in extreme cases, to complete obliteration of the lumen. It is apparent that an understanding of the source of the hyaline substance in the arteriolar wall represents a necessary requisite to understanding the pathogenesis of this lesion.

Interest in the vascular lesions of hypertensive cardiovascular disease has been revived by observations on the evolution of the arteriolar lesions in the dog following bilateral nephrectomy.[11-14] This experimental preparation has been associated, in our experience, with a high percentage of hypertension and arteriolar lesions, the latter varying because of dependence on several factors, among which has been emphasized the duration of life once the hypertensive state has ensued. Animals succumbing early have demonstrated mainly an explosive necrosis of the smooth muscle of the media of small arteries and arterioles. Animals surviving from 10 to 20 days have demonstrated necrosis of the media of small arteries and arterioles characterized by retainment of the shape of the media, the presence of pyknotic nuclear remnants, and changes which have been interpreted as the early phases of hyalinization. Occasionally in this group of animals, hyalinization of arterioles has been encountered. In those animals surviving beyond 20 days the changes in the wall of the thickened small arteries and arterioles have included total hyalinization of the wall, subendothelial hyalinization with or without medial hyalinization, fibrosis of various layers, and thickening of the internal elastic lamella. By using different stains and observation of the transitional appearances of the vascular lesions it was suggested that the hyaline material is derived from altered smooth

muscle of the media.[12-14] This concept was subsequently strengthened by the use of a battery of histochemical procedures indicating similar findings in the acute lesions of the nephrectomized dog and in the renal arteriolar lesions of "malignant hypertension" of the human.[15] In view of the demonstration of the presence of multiple substances in the walls of altered small arteries and arterioles in the hypertensive cardiovascular disease following bilateral nephrectomy of the dog and malignant hypertension of the human, it was considered worth while to study the hyaline arteriolar sclerosis of benign hypertension of the human by means of similar histochemical procedures. The results of this study are reported in the present communication. We believe they tend to characterize the source of the hyaline substance in the arteriolar sclerosis of human benign hypertension.

Methods

The renal tissues studied were obtained fresh at necropsy from four subjects whose pertinent clinical and necropsy findings are given.

Report of Cases

Case 1

A white female, 65 years old, died suddenly at home. The right kidney weighed 180 gm.; the left, 110 gm. The surface of each kidney was granular and the cortex thin. The heart weighed 500 gm. and showed coronary arteriosclerosis. Generalized arteriosclerosis was present. Microscopically, the renal lesion consisted of benign arteriolar nephrosclerosis, many small arteries showing hyalinization.

Case 2

A colored male, 51 years of age, gave a history of shortness of breath and orthopnea of 2 months' duration. His blood pressure when he was first seen in the cardiac clinic was 220/174 mm. of Hg. He was treated in the clinic for hypertensive cardiovascular disease and returned to the hospital because of mental confusion. The blood pressure at that time was 200/146. He expired shortly after admission to the hospital. At necropsy the heart weighed 520 gm. and showed a slight amount of arteriosclerosis. The kidneys weighed 150 gm. each. Their surfaces were finely granular and the capsules stripped with difficulty. The cortex was thinner than normal. Microscopically, the kidneys showed a pronounced degree of benign arteriolar necrosis, superimposed upon which was a moderate degree of acute arteriolar necrosis.

Case 3

A white female, 70 years old, had been known to have diabetes for 20 years. She had been observed for most of that period in the diabetic clinic where a diagnosis of the Kimmelstiel-Wilson syndrome was made. The blood pressure had been 150/100 mm. of Hg for many years. During the terminal admission she had diabetic acidosis with coma and cardiac failure. Deterioration was rapid. At necropsy the heart weighed 480 gm.; the right kidney, 160 gm.; the left kidney, 140 gm. Both kidneys

displayed coarsely granular surfaces with marked atrophy of the cortex. There was severe generalized arteriosclerosis. Microscopically, the renal lesions consisted of severe benign arteriolar nephrosclerosis with nodular or diabetic intercapillary glomerulosclerosis.

Case 4

A colored male, 53 years of age, entered the hospital for the fourth time with his chief complaints relating to cardiac failure. He was known to have had hypertension for 3 years, the blood pressure ranging between 190/140 and 160/120 mm. of Hg. The previous admissions had been for congestive heart failure. Demise was due to pulmonary embolism. At necropsy the heart weighed 700 gm. and showed marked subendocardial fibrosis. Generalized arteriosclerosis was prominent. Each kidney weighed 180 gm. The capsules stripped with difficulty, revealing a finely granular surface. Moderate atrophy of the cortex was present. Microscopically, the kidneys showed benign arteriolar nephrosclerosis.

The blocks of tissue were frozen in liquid nitrogen and then kept in the deep freeze until use. Before the sections were cut by the standard frozen section technique the tissue was fixed in the appropriate fixative for the histochemical procedure to be employed. The histochemical procedures employed are described in the standard texts[16,17]; minor variations in the techniques are indicated. Two sets of tissue sections were studied with each histochemical technique. One set was studied by means of the conventional frozen section technique utilizing unfixed tissues. The other set was prepared by a freezing and drying apparatus.* The lipid stains were performed on material embedded in carbowax after the freeze-drying preparation, while all other procedures were performed on paraffin-embedded tissues. In all cases entirely similar results were obtained. The freezing and drying technique afforded the advantage of preventing diffusion and of allowing the study of serial sections of the same block by a variety of procedures, thus giving continuity to the lesion in question.

The lipid components of the lesion were studied by the following procedures: oil red O, Nile blue sulfate, Schultz, Sudan black B, osmic acid, and Baker's acid hematin reaction with pyridine extraction as a control. Free aldehyde groups were demonstrated by the Schiff procedure. Sulfuric acid esters of polysaccharides were stained by the Congo red amyloid stain. Mucin was stained with Best's mucicarmine stain. Glycogen was stained by Best's glycogen stain. Alkaline and acid phosphatase were demonstrated by the method of Gomori. Free carbonyl groups were demonstrated by the method of Ashbel-Seligman.[18] Potassium was demonstrated by MacCallum's method as

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modified by Gomori.[16] Protein bound sulfhydryl groups were demonstrated by the method of Barrnett and Seligman.[19]

Results

The hyaline material of arteriolar sclerosis is illustrated in Figure 1 after use of Mallory's triple stain. After staining with Verhoeff's stain, Figure 2 shows that the elastic tissue may become fragmented and dispersed throughout the hyaline structure. All of the other figures illustrate the multiplicity of ingredients identified by the histochemical procedures and which will be discussed.

Triglycerides, fatty acids, and phosphatides were demonstrated by positive oil red O stain (Fig. 3), Nile blue sulfate (Fig. 4), and Sudan black B stain (Fig. 5). Acetone extraction failed to modify the Sudan black B stain. The fact that the predominant color in the Nile blue sulfate stain was blue seemed to indicate that the majority of the lipids were present as acidic lipids. Cholesterol and cholesterol esters were present as part of the lipid component as evidenced by the positive Schultz reaction. Although this reaction is not entirely specific for cholesterol, it appears that cholesterol and its esters account for the major portion of the color produced by the reaction. The other lipid stains, as revealed by the osmic acid and Baker's acid hematin reaction, were negative. The Schiff reaction (Fig. 6) was positive, indicating the presence of free aldehyde groups. This reaction was more intense when either frozen or frozen dried tissue was employed, suggesting that part of the positive material consisted of aldehyde groups associated with polysaccharides, mucopolysaccharides, mucoproteins and glycoproteins, glycolipids, and unsaturated lipids; possibly a small fraction resulted from the reaction of aldehyde groups associated with elastic tissue. Alcohol or acetone extraction failed to modify the reaction. The positive Congo red amyloid stain (Fig. 7) indicated the presence of sulfuric acid esters of a polysaccharide, thus offering confirmatory evidence of the presence in the lesions of polysaccharide complexes. Free carbonyl groups were present as demonstrated by the positive reaction employing the Ashbel-Seligman technique[18] (Fig. 8). Potassium was present in the lesions as demonstrated by Gomori's modification of MacCallum's method (Fig. 9). Protein-bound sulfhydryl groups were present in the hyalin as demonstrated by the method of Barrnett and Seligman[19] (Fig. 10). The lesions did not contain acid or alkaline phosphatase by Gomori's method, mucin by Best's mucicarmine stain, glycogen by Best's glycogen stain, non-spe-

cific esterase by the Nachlas and Seligman method,[20] desoxyribose nucleic acid by the Feulgen procedure, nor vitamin C.

These histochemical procedures are not considered to be quantitative. It would appear, however, that the hyaline lesions gave more intense reactions with the following procedures than normal arterioles or smooth muscle: oil red O, Nile blue sulfate, Sudan black B, Schultz', Schiff's, Congo red, and potassium. The hyaline substance gave the same reaction for free carbonyl groups and protein-bound sulfhydryl groups as normal arterioles and normal smooth muscle. All of these substances are present in the media of normal arterioles and normal smooth muscle; their apparent increment within the arteriolar lesion in some instances need not necessarily indicate a quantitative increment but may be accounted for as well by an alteration in their physicochemical state as a consequence of the process of hyalinization.

In a previous communication[15] the same battery of histochemical tests was applied to the acutely necrotic arteriole of human malignant hypertension and the necrotic arteriole of the bilaterally nephrectomized dog. In these the histochemical results were identical and with two exceptions the results reported here for the hyaline lesions are identical to those reported for the necrotic arterioles in the previous paper. The most outstanding difference consisted of the presence of acid phosphatase in the acute lesion and its absence in the hyaline lesion.

The second difference resulted from the presence of material giving a positive stain with Sudan black B in the hyaline lesion and its previously reported absence in the acute lesion. The latter difference was discovered to be due to the technique of staining. In the negative case (acute lesion) the procedure for staining was 15 minutes in duration while in the positive instance the stain was developed over a 24-hour period as suggested by Pearse.[21] When the material was restudied using the 24-hour technique, all of the lesions proved to have Sudan black B positive material. Thus the acute human and canine lesions and the hyaline arteriolar sclerotic lesions of man differed histochemically only in the presence of acid phosphatase in the former and its absence in the latter.

Discussion

From the results obtained by conventional routine stains and connective tissue stains, and the appearance of transitional changes it has been considered that the hyalin of the small arteries and arterioles in bilaterally nephrectomized dogs is derived in the main from altered necrotic smooth muscle of the media of these vessels.[11-14] It also has

been emphasized that the necrotic lesions of the small arteries and arterioles of nephrectomized dogs are similar to the necrotic lesions of arterioles in malignant hypertension of the human. The resemblance between the lesions of the dog and of man was further strengthened by the demonstration that a battery of histochemical procedures gave the same positive and negative results in these two lesions.[15] Moreover, the substances identified by the histochemical approach could be readily derived from altered smooth muscle. These observations tended to strengthen the view that altered smooth muscle is the source of the hyaline substance in the walls of arterioles.

The present observations on the typical lesion of benign nephrosclerosis, namely, benign arteriolosclerosis or diffuse arteriolar sclerosis, suggest that the hyaline substance is composed of a multiplicity of ingredients, some of which have been identified by this study. These ingredients, with one exception, are those which were found in the acute necrotic lesion of the dog following nephrectomy and in the human in malignant hypertension. The one exception is the enzyme acid phosphatase, perhaps the ingredient most eligible to disappear with time. Thus a histochemical similarity is demonstrated between necrotic smooth muscle of the media of small arteries and arterioles and the smooth acidophilic substance known as hyalin in arteriolar sclerosis. These observations suggest that the hyaline substance is not deposited from a hematogenous source, but represents the ultimate fusion of products derived from necrotic smooth muscle and that, therefore, hyalin in arteriolar sclerosis is autochthonous.

Emphasis has been placed on the cholesterol content of these regions by Baker and Selikoff[22] and of the polysaccharide content of similar experimental lesions by Masson and co-workers.[23] The lipid components of this lesion have been known for some time and have been discussed by Fishberg.[24] The present observations confirm and extend these isolated findings by pointing out that the hyaline structure contains a multiplicity of ingredients. It may be adduced that additional components may yet be identified.

The autochthonous origin of the hyalin of arteriolar sclerosis is in keeping with an outstanding feature of this lesion which has been described or depicted photographically or diagrammatically by many workers, namely, the inverse relationship between hyaline deposition and smooth muscle content of the arterial or arteriolar wall. It is common to encounter attenuation or disappearance of the smooth muscle fibers as hyalin accumulates. The demonstrations of Duguid and Anderson,[8] which were considered to support the hematogenous

origin of the hyaline substance, reveal this reciprocal relationship. Moreover, on occasions one can observe hyaline swelling of individual smooth muscle fibers of the media adjacent to a focus of hyaline deposition, a change which may be considered as an additional transitional link indicating the muscular origin of the hyaline substance.

It has been emphasized by others, namely, Moritz and Oldt,[6] that the principal location of the hyaline accumulation in arteriosclerosis is subendothelial as demonstrated by elastic tissue stains. Although there was subintimal hyaline accumulation in many of the arterioles, this hyalin was contiguous with that of the media and in these areas of continuity the elastic fibers of the elastic lamella fragmented and frequently disappeared completely. This observation offers an explanation for the accumulation of hyalin in a subendothelial location, for it appears more plausible to consider the flow of hyalin from the media into the subendothelial zone through the fragmented and broken elastic tissue than to assume a hematogenous source. This explanation is in keeping with the similar histochemical characteristics of the hyalin in both locations.

Summary and Conclusions

A histochemical characterization of the hyalin of arteriolar sclerosis of benign hypertension has demonstrated the presence of lipids, carbohydrates, free carbonyl groups, protein-bound sulfhydryl groups, and free potassium.

Since similar compounds have been identified in normal smooth muscle, it is likely that the compounds identified in the hyaline substance take their origin from the smooth muscle of the media.

An apparent increase of concentration of some of the ingredients, *i.e.*, lipids, carbohydrates, and potassium, in hyalinized arterioles as compared to normal arterioles is considered to result, in all probability, from unmasking of these substances due to an alteration in their physicochemical state during the process of hyalinization.

The suggestion is made that the subendothelial location of hyalin in some instances results from the flow of hyalin of medial origin through the fragmented internal elastic membrane to a sub-endothelial location.

The hyaline lesions of benign arteriolar sclerosis differ histochemically from the previously reported acute arteriolar lesions of malignant hypertension only in the absence of acid phosphatase.

Hyalin is considered to result from an alteration of the smooth muscle of the media of the arteriole.

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LEGENDS FOR FIGURES

FIG. 1. Case 3. Hyaline arteriole in the kidney. Mallory's triple stain. × 75.

FIG. 2. Case 4. Hyaline arteriole in the kidney. Verhoeff's elastic stain. × 75.

FIG. 3. Case 2. Lipid content of a hyaline arteriole in the kidney. Oil red O stain. × 75.

FIG. 4. Case 2. Lipid content of a hyaline arteriole in the kidney. Nile blue sulfate stain. × 75.

FIG. 5. Case 4. Lipid content of a hyaline arteriole in the kidney. Sudan black B stain. × 75.

FIG. 6. Case 1. Free aldehyde group in a hyaline arteriole in the kidney. Schiff's reaction. × 75.

FIG. 7. Case 3. Polysaccharide complexes in a hyaline arteriole in the kidney. Congo red reaction. × 75.

FIG. 8. Case 4. Free carbonyl groups in a hyaline arteriole in the kidney. Ashbel-Seligman technique. × 75.

FIG. 9. Case 2. Free potassium in a hyaline arteriole in the kidney. Gomori's modification of MacCallum's method. × 75.

FIG. 10. Case 4. Protein bound S-H groups in a hyaline arteriole in the kidney. Barrnett and Seligman's method. × 75.

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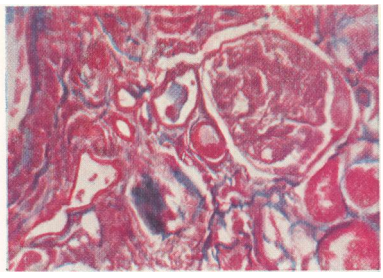

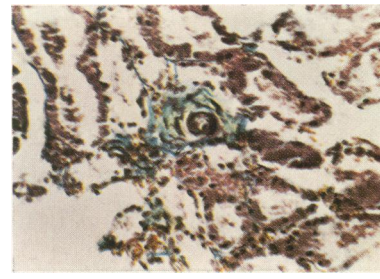

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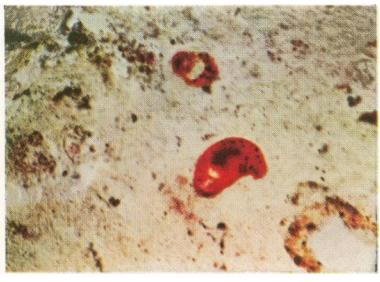

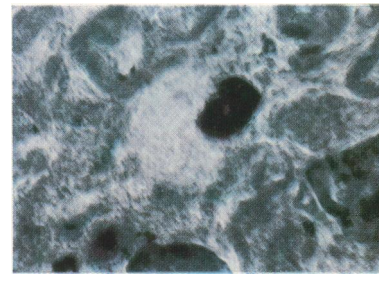

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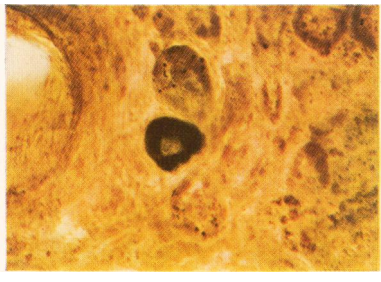

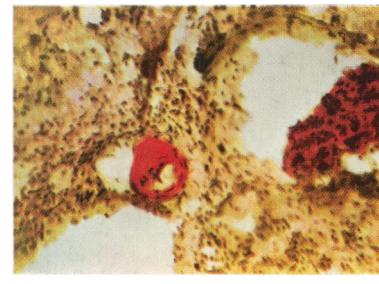

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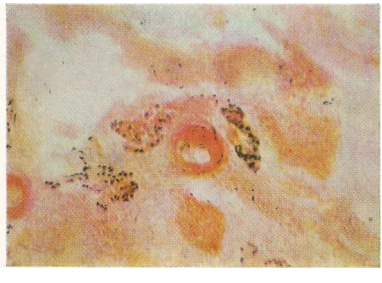

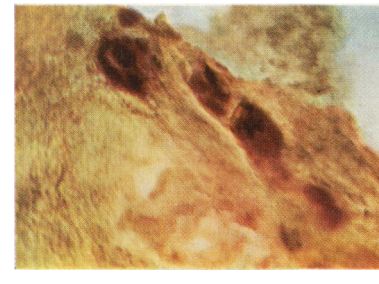

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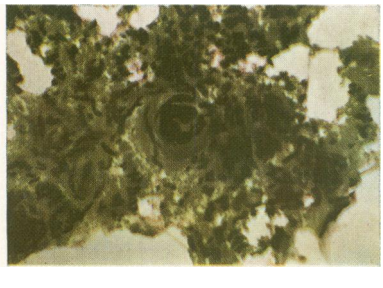

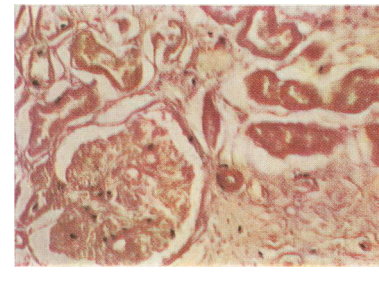

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