Vascular Pathology of Homocysteinemia: Implications for the Pathogenesis of Arteriosclerosis

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INDIVIDUALS with homocystinuria [1,2] have been found to lack normal activity of the enzyme cystathionine synthetase.[3] In many of the patients progressive arterial disease develops in childhood, frequently resulting in death from thrombosis in a vital organ.[4,5] In addition, congenital dislocation of the lenses, mental retardation, and skeletal abnormalities—eg, osteoporosis, arachnodactyly, and pectus excavatum or pectus carinatum—usually are found.[5,6] The vascular changes and other abnormalities encountered in homocystinuria have been attributed either to the metabolic effects of the elevated tissue concentrations of methionine, homocysteine, or homocystine, or to the metabolic consequences of decreased tissue concentrations of cystathionine found in the disease.[7]

In a child dying with homocystinuria, cystathioninuria, and methyl malonic aciduria, secondary to an abnormality of cobalamin (B_{12}) metabolism, arterial lesions have been discovered that resemble in a striking way many of those found in cystathionine synthetase deficiency. The vascular findings in this patient will be presented and compared with those in a patient with cystathionine synthetase deficiency. Since the enzymatic abnormalities in both disorders share certain metabolic consequences, the conclusion has been reached that an elevated concentration of homocysteine, homocystine, or a derivative of homocysteine is the common factor leading to arterial damage. The possible role of elevated concentrations of homocysteine or its derivatives in the pathogenesis of arteriosclerosis in individuals free of known enzyme deficiencies will be discussed and interpreted with particular reference to the findings in experimentally produced arteriosclerosis.

Case Material

Patient 1: Abnormality of Cobalamin Metabolism with Homocystinuria, Cystathioninuria, and Methylmalonic Aciduria

A male child born at term failed to gain weight because of vomiting,

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Supported in part by Grant-in-Aid 924 from the Greater Boston Chapter of the Massachusetts Heart Association.

Accepted for publication April 4, 1969.

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lethargy, and poor feeding. He was found to have aspiration pneumonitis without septicemia; traces of urinary and gastrointestinal hemorrhage; and persistent normocytic, normochromic anemia. Following an episode of respiratory and metabolic acidosis, he expired at the age of 7½ weeks after several episodes of convulsions and cardiac arrest.

During the course of his illness, large quantities of homocystine, cystathionine, and homocysteine-cysteine disulfide were discovered in plasma and urine, as reported in detail elsewhere.[8] Plasma homocystine concentration was 1.9 mg/100 ml, and urinary homocystine excretion was 5.1 mg/24 hr. (Homocystine is not found in normal plasma or urine by the methods used.) Plasma cystathionine concentration was 0.20 mg/100 ml, and urine cystathionine excretion was 2.0 mg/24 hr. The plasma methionine concentration was very low (0.07 mg/100 ml). In addition, methylmalonic acid was excreted in large quantities (17.6–19.3 mg/24 hr) both before and after treatment with Vitamin B_{12} (cyanocobalamin). The activity of the cobalamin-dependent enzyme system, N^5-methyltetrahydrofolate-homocysteine methyl transferase, was found to be reduced in postmortem liver and kidney, as compared to that in control tissues; the elevation of homocystine and cystathionine, as well as the depletion of methionine, were attributed to this reduced activity. Assays for several other sulfur amino acid enzymes, including cystathionine synthetase, cystathionase, methionine-activating enzyme, and betaine homocysteine methyl transferase, were all within the normal range. A disorder of cobalamin metabolism is indicated both by the very reduced concentration of deoxyadenosyl cobalamin coenzyme found in the liver and kidney of this patient and by the excretion of methylmalonic acid.

The necropsy was performed 1 hr post mortem, and tissue samples were frozen for enzyme assays and fixed in formalin for pathologic study. Sections were stained with hematoxylin and eosin (H&E); and Verhoeff elastic tissue with Van Gieson counterstain, periodic acid-Schiff, phosphotungstic acid-hematoxylin, toluidine blue, and alcian blue. Representative blocks also were embedded in Epon; 1-μ sections were stained with Giemsa for light microscopy, and selected tissues were examined by electron microscopy. The sections were compared with suitable age-matched controls.

Aside from the cardiovascular findings, to be described in detail, the necropsy revealed bilateral bronchopneumonia; moderately fatty liver; gastric mucosal atrophy with cystic change, and squamous and intestinal metaplasia; and myeloid hyperplasia without megaloblastic maturation. The brain was small, and there was diffuse and focal soften-

ing of the white matter which was not interpreted as an infarct. Detailed reports of the clinical and metabolic[9] as well as the biochemical findings[8] are presented elsewhere.

Patient 2: Cystathionine Synthetase Deficiency With Homocystinuria

In a case reported in 1933,[10] an 8-year-old retarded male child with dislocated lenses was presented with a 4-day history of vomiting, headache, coma, and spastic hemiparesis on the left side. The diagnosis at necropsy was arteriosclerosis of the internal carotid arteries and carotid thrombosis with a massive recent infarct of the right cerebral hemisphere. Recently, the brother and two nieces of this patient have been discovered to have homocystinuria and cystathionine synthetase deficiency.[11] In case 2, the findings of retarded mental development, congenital dislocation of the lenses, and death from vascular thrombosis in childhood also suggest cystathionine synthetase deficiency; the pathologic findings, to be described, support the diagnosis. Therefore, this patient is presented as a presumptive case of cystathionine synthetase deficiency with homocystinuria. Sections from several tissues stained with hematoxylin and eosin, or Verhoeff elastic tissue with Van Gieson counterstain, are available for study. The limited number of available tissue blocks were re-embedded, cut, and stained with H&E, Verhoeff elastic tissue with Van Gieson counterstain, toluidine blue, or alcian blue for further study. The sections were compared with suitable age-matched controls and with selected sections from three patients with cystathionine synthetase deficiency reported in the literature.[5,6] The arterial changes found in Patient 2, involving both large and small arteries, were very similar to those present in the three patients with proven homocystinuria.

Pathologic Findings

Patient 1

No gross lesions or occlusions were found in the cardiovascular system, nor were infarcts found in the various organs. However, extensive focal microscopic alterations involved large, medium-sized, and small arteries in many organs of the body. A branch of the renal artery (Fig 1) contained a prominent fibrous intimal plaque formed from focal splitting, fraying, and disruption of the internal elastic membrane, and proliferation of intimal loose fibrous connective tissue. A branch of the left coronary artery (Fig 2) was narrowed by a proliferation of intimal and medial loose vacuolated fibrous tissue, associated with fraying and destruction of the internal elastic membrane. In Fig 3 a main branch of

the pulmonary artery is shown to be narrowed by a moderate intimal proliferation of loose fibrous connective tissue. There was no change in the underlying internal elastic membrane and medial elastic tissue of the pulmonary artery. The aorta was normal, and no increase in metachromatic material was observed with special stains. The veins in the various organs were normal.

Lesions in the medium-sized and small arteries were present in the adrenals, pancreas, thyroid, testis, heart, lung, kidney, liver, lymph nodes, thymus, stomach, small intestine, and brain. The number of affected arteries varied from a small proportion to a majority of those observed in the various sections. As exemplified by the small artery in the gastric submucosa (Fig 4), many small arteries were surrounded by increased perivascular loose connective tissue consisting of collagen bundles; fine, irregular elastic fibers; and increased numbers of fibroblasts. A small pancreatic artery was narrowed by an irregular, folded focally discontinuous internal elastic membrane; enlarged vacuolated endothelial cells; and moderate thickening of the media. The arterioles from the pancreas of this patient (Fig 5A) and from an age-matched control (Fig 5B) demonstrate the degree of proliferation of loose perivascular connective tissue, as well as the prominent, vacuolated endothelial cells in many arterioles in the patient, compared to the control. A small artery from the testis was altered focally by disruption of the intima and media, and by swelling and hyperplasia of the endothelial cells, associated with increased loose perivascular connective tissue. A few small fragments of dense basophilic material resembling nuclear debris were present in the media and adventitia of that vessel.

The walls of some of the small arteries of the cerebral cortex and centrum semiovale were thickened by proliferation of endothelial cells and perivascular spindle-shaped cells. The walls of many small arteries and arterioles in the centrum semiovale, which were associated with microscopic hemorrhages, were disrupted focally and partially replaced by accumulations of eosinophilic, homogeneous, acellular, weakly phosphotungstic acid-positive material (Fig 6).

Maturation of the glomeruli was within the range of normal for a neonatal kidney. In a few of the glomerular capillary tufts, there was a moderate increase in mesangial matrix, which was weakly PAS-positive, associated with some enlargement and a moderate increase in numbers of endothelial and mesangial cells. There was no evidence of inflammation, necrosis, thickening of basement membranes, or intracapillary thrombi within glomeruli. A few tubules and the Bowman's spaces of a few glomeruli contained erythrocytes.

Electron microscopic examination confirmed the presence of increased amounts of normal collagen and elastic tissue about small arteries in many organs. An intimal plaque in a medium-sized artery was found to consist of loose fibrous tissue comparable to that found in typical arteriosclerotic fibrous intimal plaques.

Patient 2

Some aspects of the cardiovascular findings are presented and discussed in a previously published report.[10] In summary, the pertinent gross findings are confined to the carotid arteries and brain. The walls of both carotid arteries were thickened, and there was severe narrowing of the lumens. The right carotid artery was further narrowed to almost complete occlusion by organized thrombus. The right half of the arterial circle of Willis contained a recent thrombus, and the right cerebral hemisphere was softened and pale, the result of an extensive early infarction. There was no gross evidence of disease within the heart, aorta, pulmonary artery, venae cavae, or other major vessels, except for the carotid arteries.

As illustrated in Fig 7, the changes in the right internal carotid artery consisted of an organized mural thrombus almost completely occluding the lumen; intimal thickening by a marked proliferation of loose fibrous connective tissue; disorganization and fibrosis of the media; loss of elastic tissue of the media; and reduplication, fraying, thickening, and discontinuity of elastic fibers of the internal elastic membrane. A toluidine blue stain showed small amounts of metachromatic ground substance within the media and intimal fibrous tissue.

Additional study of the re-embedded tissues revealed widespread focal alterations in the medium-sized and small arteries in the thymus, adrenal, kidney, heart, and lymph nodes. In many medium-sized arteries there was focal narrowing of the lumen by intimal fibrosis, associated with splitting, irregularity, and focal discontinuity of the internal elastic membrane (Fig 8 and 9). As exemplified by the findings in the kidney and adrenal glands (Fig 9 and 10), many small arteries and arterioles were surrounded by a moderate proliferation of fibrous tissue containing increased numbers of fibroblasts, collagen fibers, and thin, irregular elastic fibers; the media of some of these vessels was moderately thickened by hyperplastic smooth-muscle cells.

In the kidney, many of the glomeruli were slightly to moderately hypercellular because of increased numbers of mesangial and endothelial cells; marked red cell congestion was present in many of the glomerular capillary tufts. One section of the aorta was normal. Moder-

ate centrilobular fatty change was present in liver parenchymal cells, and extensive acute bronchopneumonia was found.

Discussion and Conclusions

The severe arterial changes found in a patient with cystathionine synthetase deficiency and those encountered in a patient with abnormal cobalamin metabolism with homocystinuria, cystathioninuria, and methylmalonic aciduria were similar in many important respects. First, the lesions were focal and involved numerous large, medium-sized, and small arteries in many organs. Second, the lumens of many large and medium-sized arteries were narrowed by focal intimal and medial fibrosis, often associated with fraying and discontinuity of the internal elastic membranes. Third, there was focal proliferation of perivascular connective tissue surrounding many small arteries, with an increase in numbers of fibroblasts, collagen bundles, and small elastic fibers. Finally, slight-to-moderate thickening of the media and prominence of the internal elastic membranes were present in some medium-sized and small arteries. Significant venous disease was not found in either case.

Some differences were found in the distribution, extent, and apparent age of the arterial lesions in the two patients. In the small arteries of the brain of Patient 1, focal disruption of the wall was associated with the deposition of varying amounts of amorphous eosinophilic fibrinoid material and focal hemorrhage (Fig 6). In the small arteries of the pancreas, kidney, adrenal, and intestine, there were a few small fragments of dense basophilic material resembling nuclear debris within media and adventitia, apparent edema within the adventitia, and swelling of the endothelial cells (Fig 5A). These changes were not found in Patient 2, nor have they been described in other cases of cystathionine synthetase deficiency reported in the literature. The intimal and medial lesions in large arteries in Patient 1 did not narrow the lumen as severely as those observed in Patient 2, and there was no evidence of thrombosis of large arteries, such as the carotid thrombosis found in Patient 2. The focal glomerular changes found in Patient 1 have not been reported in cystathionine synthetase deficiency, although some of the glomeruli in Patient 2 were found to contain a slight-to-moderate increase in the size and number of endothelial and mesangial cells. The factor interpreted as accounting for these differences in the vascular pathology in the two patients is that the child with homocystinuria and cystathioninuria survived only 2 months after birth. The arterial lesions of Patient 1 were probably at an earlier stage of development than those found in the patient with cystathionine synthetase deficiency, who survived 8 years.

Presumably because cystathionine synthetase deficiency blocks the condensation reaction of homocysteine with serine, tissue concentrations of homocysteine, homocystine, and methionine are elevated, and cystathionine concentrations are decreased in these individuals. In Patient 1 (with abnormal cobalamin metabolism and reduced N^5-methyltetrahydrofolate homocysteine methyl transferase activity) the concentration of methionine was reduced, and the concentrations of homocystine and cystathionine were elevated—presumably because of a deficient rate of methylation of homocysteine. Since prominent arterial damage, sharing many important features, has been found in both of these diseases, the conclusion is reached that the vascular alterations are secondary to the metabolic effects of elevated tissue concentrations of homocysteine, homocystine, or derivatives of homocysteine, which are found in both diseases.

If vascular damage were produced by the metabolic effect of elevated concentrations of methionine but not by homocysteine or its derivatives, arterial disease would not be expected to have occurred in Patient 1, in whom low methionine concentration was found. Also, vascular disease has not been found in children dying with hypermethioninemia associated with tyrosinemia and islet cell hyperplasia,[12,13] even though large amounts of methionine are excreted by these children. If vascular damage were produced by the effects of deficient concentrations of methionine in tissue, arterial disease would not be expected to occur in cystathionine synthetase deficiency in which there is elevated methionine concentration.

If an elevated concentration of cystathionine were the only factor that produced vascular damage, then patients with cystathionine synthetase deficiency would not be expected to develop arterial disease, since cystathionine concentrations are deficient in these patients. Furthermore, in patients with cystathionase deficiency, large quantities of cystathionine are excreted, and the cardiovascular system has been reported to be free of disease at necropsy.[14,15] If reduced cystathionine concentration was the only factor producing arterial damage, the vascular findings in Patient 1 with increased cystathionine concentration would have to be explained in some other way. For example, the vascular disease in Patient 1 might possibly be secondary to some unrelated effect of abnormal cobalamin metabolism. The effect of elevated methylmalonic acid concentration cannot explain the vascular disease, however, since patients with methylmalonyl CoA isomerase deficiency, who excrete large quantities of methylmalonic acid, have been reported to be free of arterial damage at necropsy.[16-18] Since the

conclusions regarding the importance of homocysteine in producing arterial damage are based in part on the results in Patient 1, it would be desirable to document these arterial findings in additional patients comparable to Patient 1.

Since arterial damage is found in these two patients with enzymatic abnormalities involving homocysteine metabolism, what is the significance of elevated concentrations of homocysteine and homocysteine derivatives in the pathogenesis of arteriosclerosis in individuals free of known enzymatic deficiencies? Is it possible, for example, that in patients with hereditary, dietary, environmental, or other predisposition to arteriosclerosis—such as that observed in those who have diabetes, hypothyroidism, hypertension, radiation injury, or who smoke cigarettes —vascular damage and fibrous arterial plaques develop as a result of elevated concentrations of homocysteine or homocysteine derivatives? Although the current emphasis in the field of the pathogenesis of arteriosclerosis is on the importance of dietary fat and the stability of the various plasma lipid fractions on the development of lipid plaques and the conversion of fibrous plaques to lipid plaques, considerable opinion and evidence can be found in the literature that vascular damage, similar to that found in these patients with homocysteinemia, is the initial pathogenic factor in arteriosclerosis, and that lipid deposition is a secondary complication of the primary vascular alteration.

Unfortunately, tissue or plasma homocysteine concentrations have not been studied either in patients with a predisposition to arteriosclerosis or in animals with experimentally induced arteriosclerosis. However, as a result of the findings presented in this report, several of the important experimental studies of arteriosclerosis can be interpreted as examples of metabolic alterations in which elevated tissue concentrations of homocysteine would be expected to be associated with arterial damage.

After the discovery of the lipotropic effect of choline and related methyl donors,[19] it was shown that increased amounts of dietary choline delayed or prevented the development of atherosclerosis in cholesterol-fed rabbits,[20] and that choline deficiency led to vascular damage and arteriosclerosis in rats.[21] The fibrous intimal and medial lesions in the aorta and large arteries found in these choline-deficient rats are very similar to those observed in the two patients presented in this report. Also, branches of the coronary arteries of the choline-deficient rats were found to be surrounded by an adventitial proliferation of loose fibrous tissue similar to that found around many small and medium-sized arteries of the two patients with homocysteinemia. Aside from the

lipotropic effects of choline on fat metabolism, increased amounts of dietary choline might be expected to reduce homocysteine concentration in the tissues by increasing the rate of transmethylation of homocysteine to methionine by the methyl groups of choline. Consequently, decreased arterial damage would be expected during the experiment because of the decreased endogenous homocysteine concentration. Dietary choline deficiency might be expected to increase homocysteine concentration by reducing the rate of transmethylation of homocysteine to methionine, leading to accelerated vascular damage and arteriosclerotic lesions.

The relationship of sulfur amino acid metabolism to experimental arteriosclerosis is further demonstrated by the finding that cholesterol-fed hypothyroid rats develop aortic lesions only if sodium cholate is present in the diet.[22] Dietary cholate is known to increase the excretion of sulfur reserves by conjugation with taurine.[23] The tissue concentration of homocysteine is not known under these conditions, but mobilization of sulfur may be accompanied by homocysteinemia. In another experiment autoclaved soy bean meal, which is known to be deficient in methionine, was used as a sole protein source for monkeys on a high-cholesterol diet, and arteriosclerosis was found to develop in the animals.[24] The concentration of homocysteine in these animals has not been determined, but it is possible that the composition of the dietary protein produced homocysteinemia by increasing the rate of methionine synthesis from dietary homocystine, cystathionine, cystine, or other related sulfur amino acids.

The puzzling observation that pyridoxine-deficient monkeys develop arteriosclerotic lesions [25] can be explained by assuming that an elevated homocysteine concentration produced by pyridoxine deficiency led to initial vascular damage in these animals. In man [26] and in certain strains of rats,[27] cystathionine excretion is observed when a pyridoxine-deficient diet is employed. Pyridoxine is a co-factor both for cystathionine synthetase [28,29] and for cystathionase,[30] and it is possible that pyridoxine deficiency produced elevated homocysteine concentration in the animals, leading to arterial damage and arteriosclerosis.

The effect of increased concentrations of homocysteine (which is a key intermediate in sulfur amino acid metabolism normally present in very low concentration), or of derivatives of homocysteine, on sulfated polysaccharide, collagen, and elastic tissue synthesis and degradation has not been studied experimentally. The metabolic effects of homocysteine that lead to arterial damage are obscure, and the observations presented in this report suggest a promising area for future research

in the pathogenesis of arteriosclerosis. If the rates of the various reactions and the physiologic factors that determine endogenous homocysteine concentrations are understood sufficiently in patients with diseases predisposing to arteriosclerosis, therapeutic agents or dietary manipulations could be designed to prevent elevated homocysteine concentration, thereby delaying or preventing the onset of arterial damage.

Summary

The arterial lesions discovered in a child who has an abnormality of cobalamin metabolism resulting in homocystinemia, cystathioninemia, and methylmalonic acidemia, resemble in a striking way many of those lesions found in patients with cystathionine synthetase deficiency with homocystinemia. Since the two disorders of sulfur amino-acid metabolism both result in elevation of homocysteine concentration, the arterial damage found in association with both diseases is attributed to the metabolic effects of increased concentrations of homocysteine, homocystine, or a derivative of homocysteine. The implications of this finding for the pathogenesis of arteriosclerosis in individuals free of known enzyme deficiencies are discussed and interpreted with particular reference to the findings in experimentally induced arteriosclerosis.

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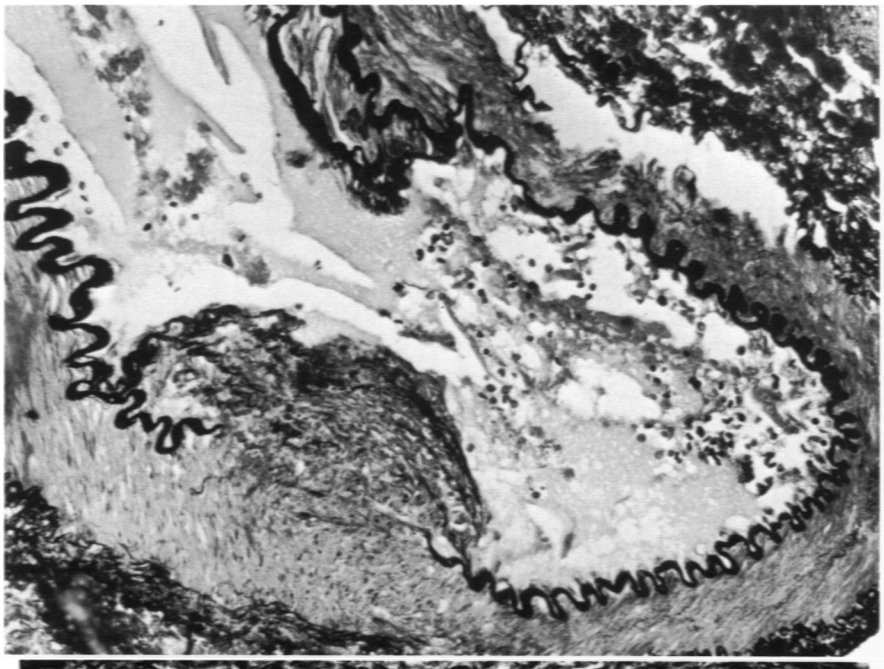
The advice and criticism by Dr. R. E. Scully; the electron microscopic examination of tissues of Patient 1 by Dr. J. B. Caulfield; and the disclosure prior to publication of the metabolic and enzymatic findings in Patient 1 by Dr. H. L. Levy are gratefully acknowledged. Also, the author wishes to thank Dr. N. A. J. Carson and Dr. A. D. Pollack for permitting him to examine sections from published cases of homocystinuria, and Dr. V. E. Shih for disclosure prior to publication of the genetic and metabolic findings in the family of Patient 2.

Legends for Figures

Fig 1. Patient 1. Fibrous intimal plaque in branch of renal artery is formed by splitting, fraying, and disruption of internal elastic membrane, and focal intimal and medial proliferation of loose fibrous connective tissue. Verhoeff-Van Gieson. × 160.

Fig 2. Patient 1. Branch of coronary artery is narrowed by proliferation of intimal fibrous tissue, associated with focal fraying and irregularity of internal elastic membrane. Verhoeff-Van Gieson. × 110.



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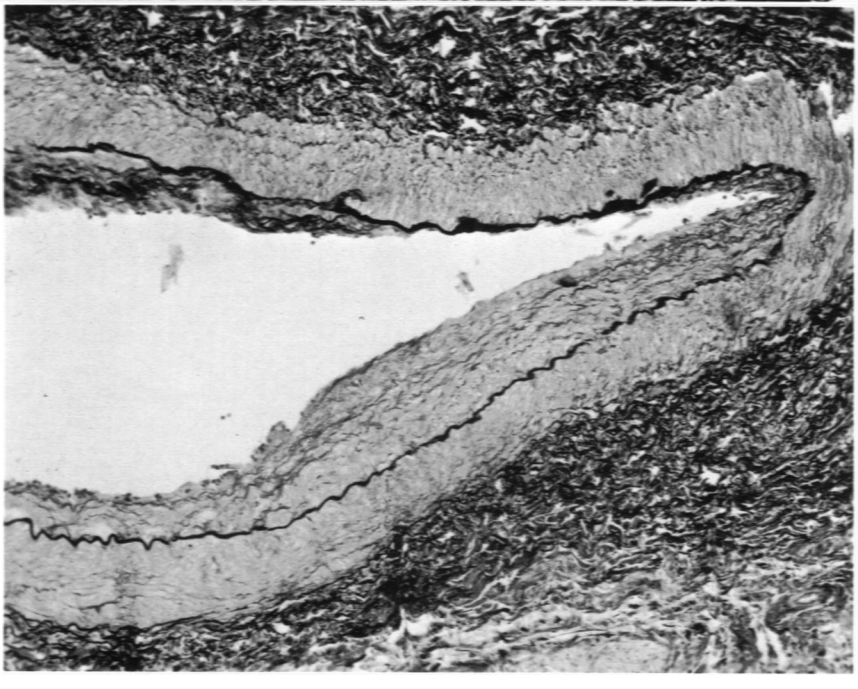

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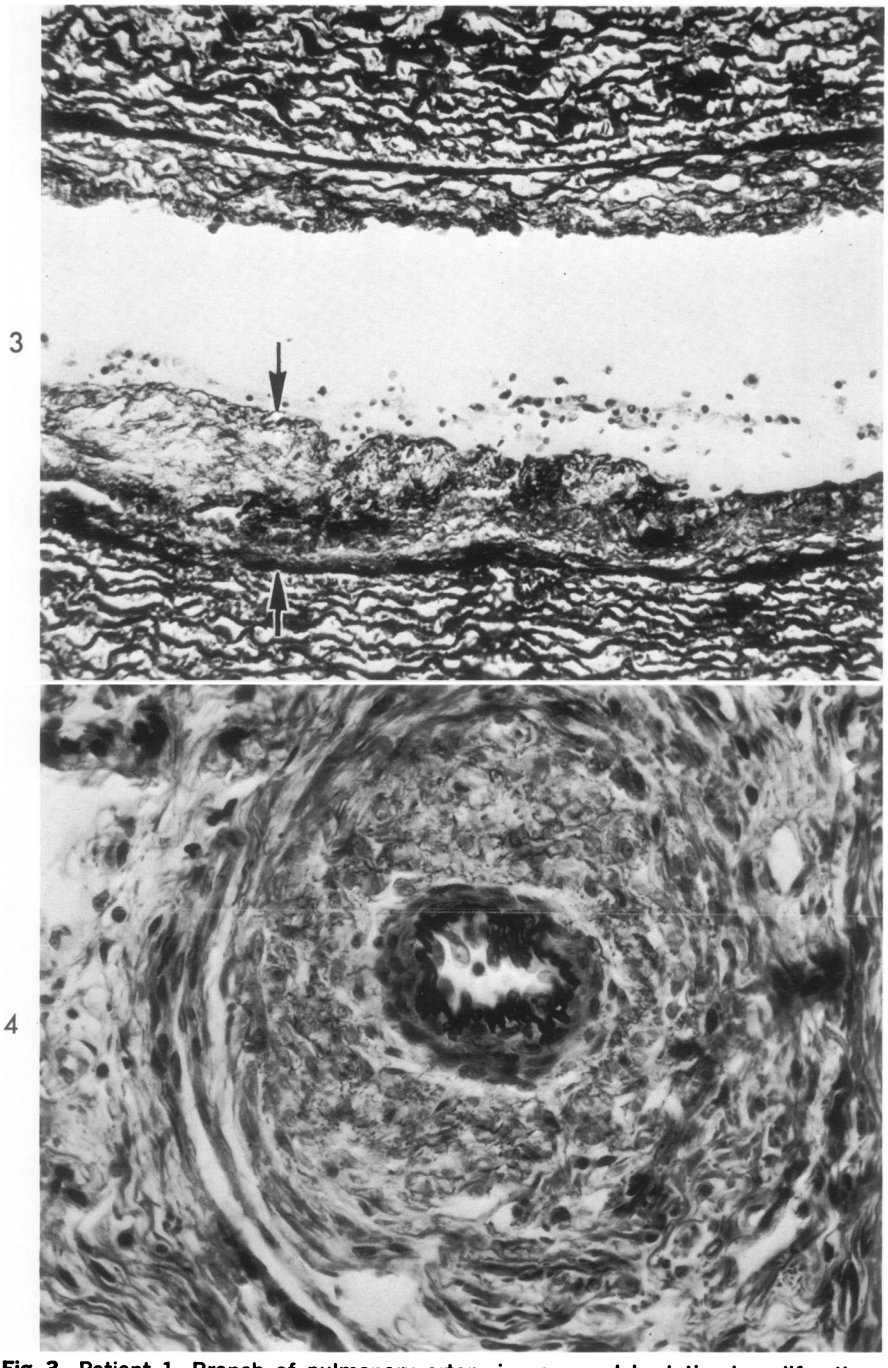


Fig 3. Patient 1. Branch of pulmonary artery is narrowed by intimal proliferation of loose fibrous connective tissue demonstrated between the arrows. Verhoeff-Van Gieson. × 210.

Fig 4. Patient 1. Small submucosal artery from stomach is surrounded by prominent perivascular connective tissue consisting of collagen fibers, small irregular elastic fibers, and fibroblasts. Verhoeff-Van Gieson. × 400.

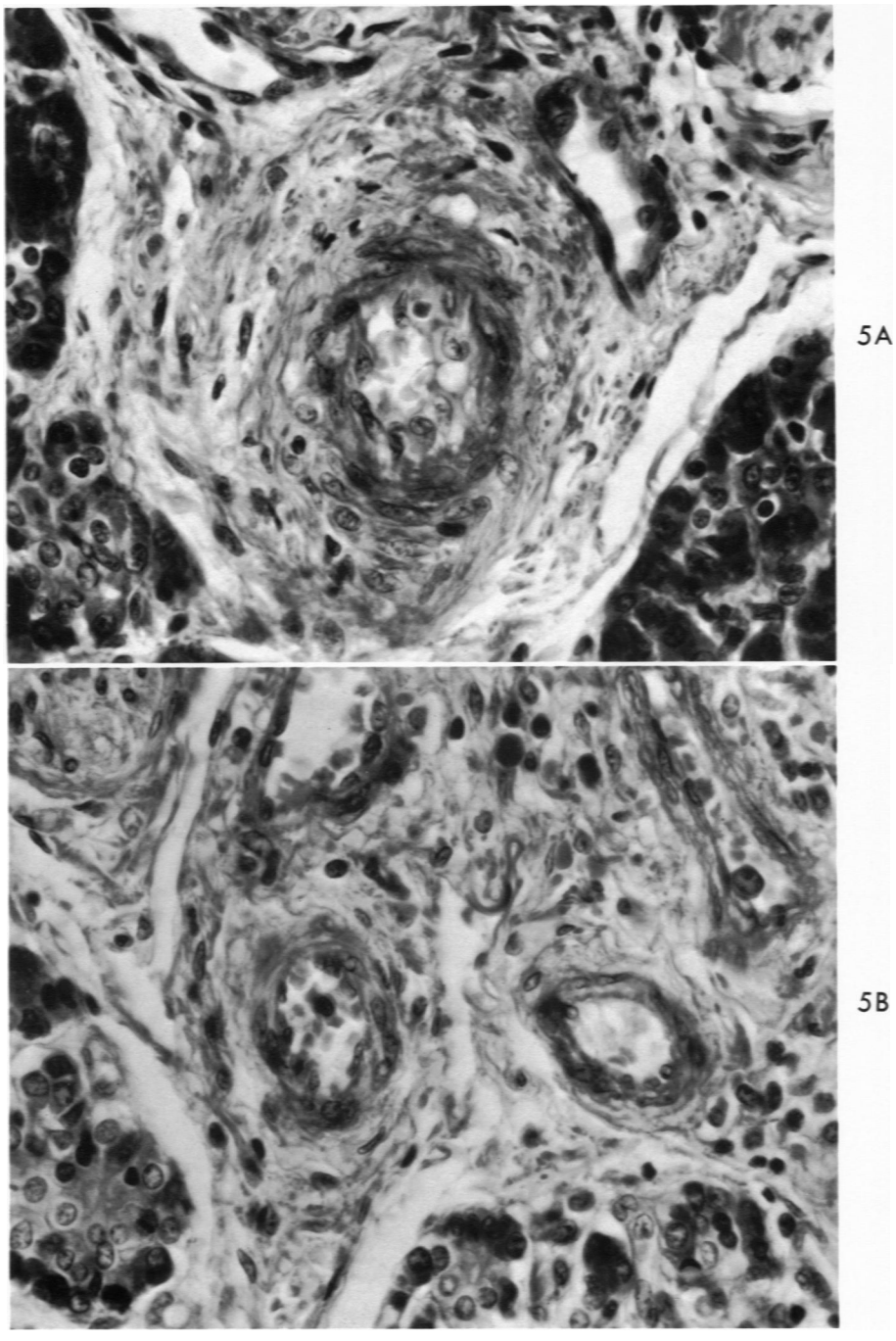


Fig 5A. Patient 1. Pancreatic arteriole is narrowed by swollen, vacuolated endothelial cells and increased numbers of spindle-shaped cells of the media. Adventitia is thickened by loose fibrous connective tissue containing a few scattered dense basophilic fragments resembling nuclear debris. Hematoxylin and eosin. × 530.

Fig 5B. Age-matched control. Normal arterioles of pancreas are to be contrasted with arterioles in Fig 5A. Hematoxylin and eosin. × 530.

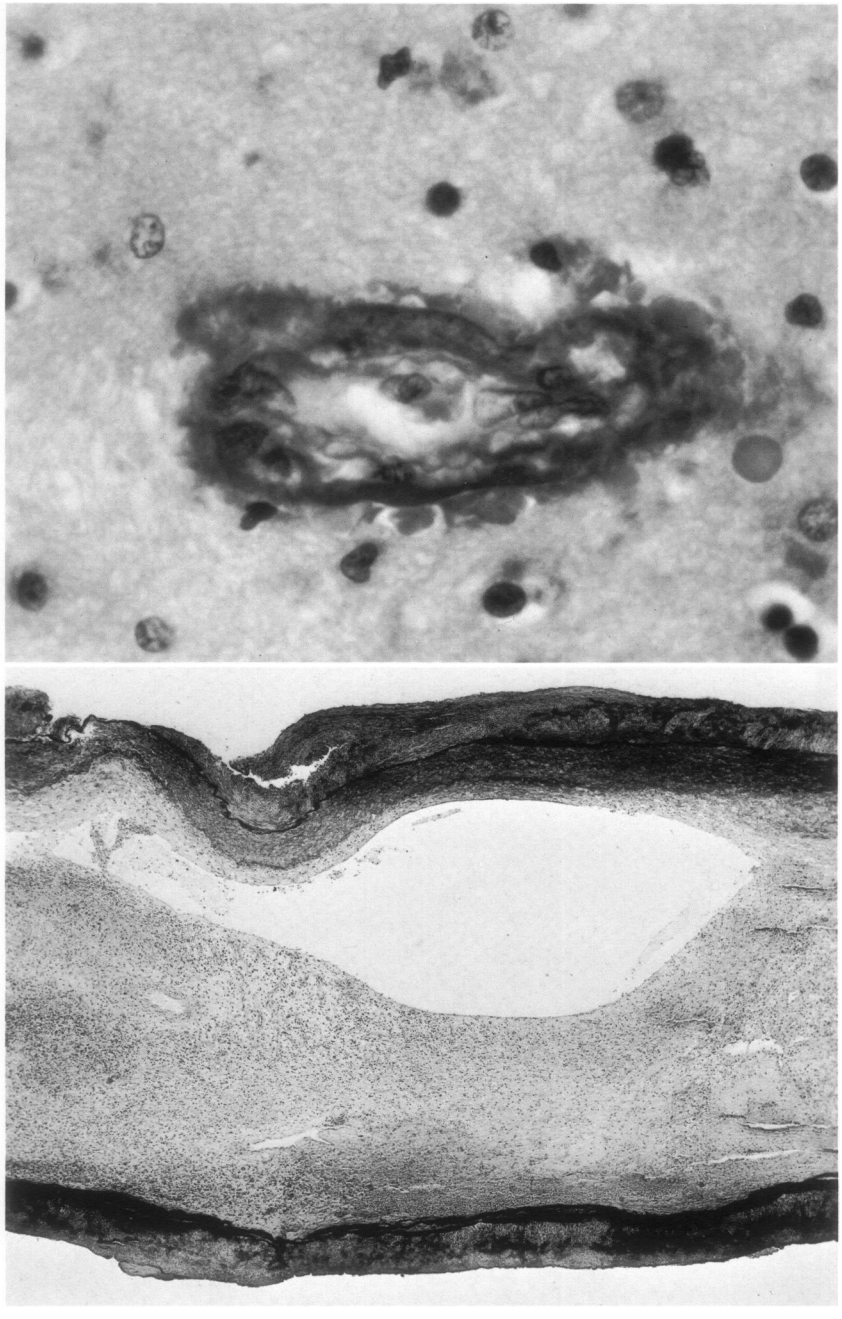
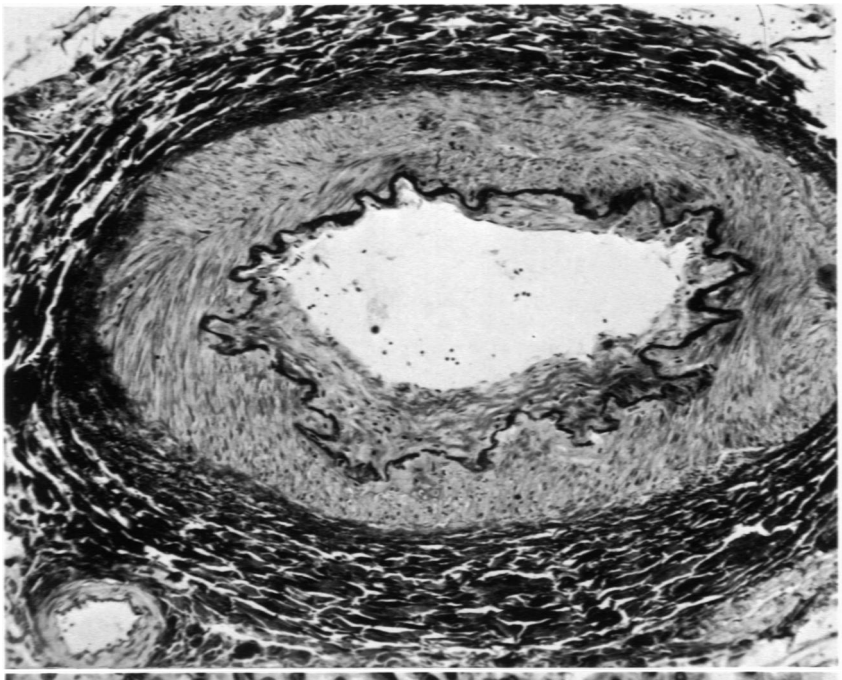


Fig 6. Patient 1. Cerebral arteriole is narrowed by enlarged endothelial cells, and the wall is disrupted and partially replaced by eosinophilic, homogeneous acellular fibrinoid material. A few extravasated erythrocytes surround the vessel. Hematoxylin and eosin. × 770.

Fig 7. Patient 2. Lumen of right common carotid artery is severely narrowed by intimal fibrosis and an adherent organizing mural thrombus. Elastic fibers of internal elastic membrane are frayed, and there is fibrosis of the media with loss of elastic fibers. Verhoeff-Van Gieson. × 38.



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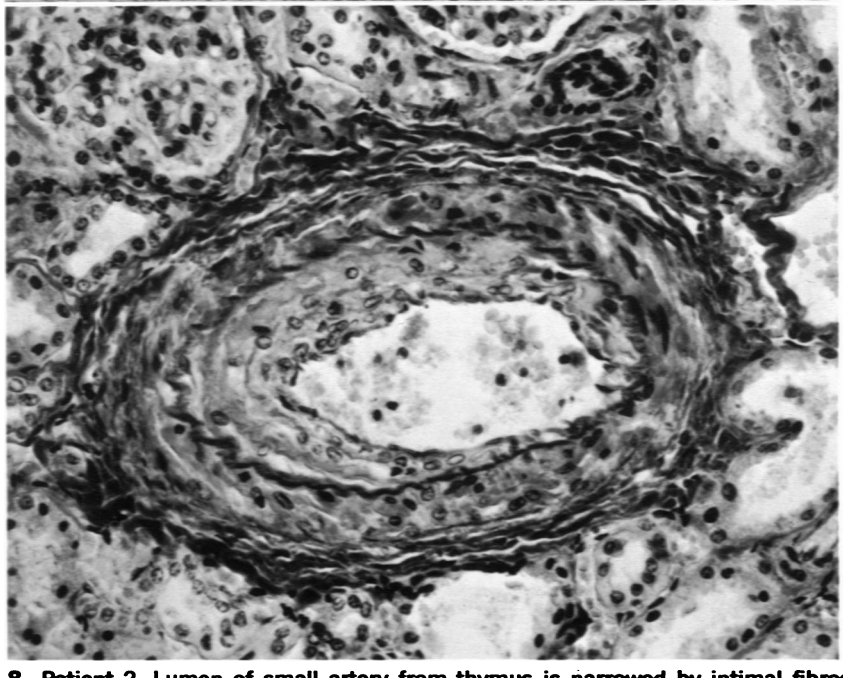

9

Fig 8. Patient 2. Lumen of small artery from thymus is narrowed by intimal fibrosis, associated with irregularity and focal discontinuity of the internal elastic membrane. Verhoeff-Van Gieson. × 120.

Fig 9. Patient 2. Lumen of small artery from kidney is narrowed by marked intimal fibrous proliferation, and there is increased adventitial fibrous tissue. Verhoeff-Van Gieson. × 290.

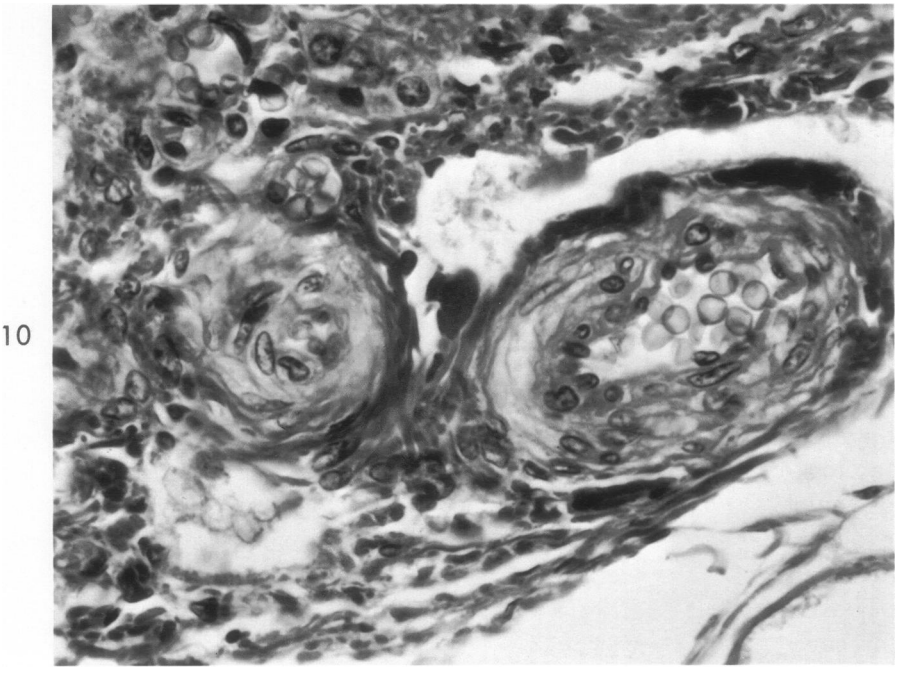

Fig. 10. Patient 2. Walls of two adrenal capsular arterioles are moderately thickened by increased numbers of spindle-shaped cells and collagen fibers. Hematoxylin and eosin. × 630.