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41. We wish to thank Miss Thérèse Fournier for her technical assistance.
42. Since this paper was submitted, one of the authors has undertaken a prospective study in a maternity hospital; 1,000 women will be sampled. The detection of pathological milks is done with a stained reaction using Nile blue (Luzeau R., Levillain P., Odièvre M., and Lemonnier A.: Dépistage des laits maternels inhibiteurs de la glucuroconjugaison de la bilirubine par une réaction colorée. Arch. Franç. Pédiat., *30:* 573 (1973)).The study's purpose is to see whether some inhibitory milks will be found in the absence of jaundice.
43. Requests for reprints should be addressed to: A. Foliot, M.D., Unité de Recherches de Biologie Animale et Techniques Experimentales, INSERM, U-36, 17 rue de Fer-a-Moulin, 75005 Paris (France).
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Homocystinuria
kidney
liver

5,10-methylenetetrahydrofolate reductase deficiency
plasma
urine

Morphologic Studies in a Patient with Homocystinuria due to 5,10-Methylenetetrahydrofolate Reductase Deficiency

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Extract

Biochemical and morphologic studies on a patient with homocystinuria due to a deficiency of 5,10-methylenetetrahydrofolate reductase (EC. 1.1.1.68) were performed.

The concentrations of homocystine in the patient's plasma and urine were 2.97 μmol/dl and 44.67 μmol/24 hr, respectively. Activities of 5,10-methylenetetrahydrofolate reductase (expressed as nanomoles of formaldehyde formed per hr per mg of protein) in cultured skin fibroblasts and liver tissue were 0.53 (control: 5.14) and 0.00 (control: 13.80), respectively.

The major abnormalities were found in the arterial bed, consisting of intimal hyperplasia, fragmentation, and disruption of elastic lamellae and subcellular changes in the endothelial cells. Extensive thrombosis was observed. The brain and the liver also showed widespread pathologic changes. In the former, neuronal loss and cellular damage were prominent and extensive. Diffuse demyelination with moderate astrocytosis was found; but demyelination was out of proportion to the vascular changes. Hirano bodies in the cortical neurons and crystalline and lamellar bodies in the Purkinje cells were observed. In the liver, there were fatty change and mild to moderate portal fibrosis. Bizarre, giant mitochondria and membrane-bound multivesicular bodies were found. Mild pathologic changes were also observed in the striated muscles and the kidneys. Focal fragmentation, disruption, and smearing of the Z discs and disorganization of the myofilaments were found in the skeletal muscles. The kidneys showed shrunken glomeruli, thickened basement membranes, and swelling of epithelial as well as endothelial cells.

Speculation

The morphologic abnormalities under light or electron microscopy in this patient with 5,10-methylenetetrahydrofolate reductase deficiency were strikingly similar to those reported in patients with cystathionine-β-synthase deficiency and with N^5-methyltetrahydrofolate homocystine methyltransferase deficiency. The common denominator in all these disorders is homocystinemia. It is postulated that the widespread vascular lesions are produced by the "toxic" effect of homocystine and that the pathologic changes in other organs are the result of ischemia and thrombosis as well as a possible direct effect of homocystine.

Homocystinuria may be due to a deficiency of cystathionine-β-synthase, N^5-methyltetrahydrofolate homocystine methyltransferase (or abnormal vitamin B_{12} metabolism) (30), 5,10-methylenetetrahydrofolate reductase, and the administration of 6-azauridine triacetate (14). Clinically, patients with cystathionine-β-synthase deficiency have ectopia lentis, skeletal deformities, marfanoid features, thromboembolic tendency, and mental retardation (1). In patients with deficiency of N^5-methyltetrahydrofolate homocystine methyltransferase and 5,10-methylenetetrahydrofolate reductase, ectopia lentis and severe skeletal abnormalities have not been observed (4, 10, 16). Concurrent biochemical and pathologic studies in the various types of homocystinuria may reveal similarities or differences which may provide insight in the pathogenesis of the observed abnormalities.

This communication records the first ultrastructural study in a patient with 5,10-methylenetetrahydrofolate reductase deficiency.

MATERIALS AND METHODS

The subject of this study was a 10-year-old Caucasian female who had delayed psychomotor development, severe mental retardation, genu valgum, and mild spastic paraparesis. Detailed ophthalmologic examination on several occasions did not show any abnormality. The proportion of her upper and lower segments was normal as were her upper limbs and fingers. X-ray showed no skeletal deformity. She was admitted to the University of Illinois Hospital at a terminal stage, with respiratory difficulties, cyanosis, seizures, and semicoma. Death occurred within 24 hr of admission and autopsy was performed within 1 hr after death.

She had three mentally retarded sisters with similar clinical illness, four normal brothers, and three normal sisters. Her clinically normal parents were of Irish ancestry and no consanguinity was recorded.

Two of the affected sisters died in other hospitals without a definitive diagnosis and in one of them the autopsy showed cerebral thrombosis, hydrocephalus, and disseminated focal demyelination.

Blood, urinary, and tissue amino acids were determined by ion exchange chromatography as previously described (31, 33). The methods of Mudd and his associates (22, 23) were used for the assay of cystathionine-β-synthase, N^5-methyltetrahydrofolate homocystine methyltransferase, and 5,10-methylenetetrahydrofolate reductase. Tissues obtained at autopsy were immediately frozen and maintained at −40° until enzyme assays.

Tissues for light microscopy were fixed in 3.7% formaldehyde, sectioned, and stained with hematoxylin-eosin, Masson trichrome, elastica (Hart), Weil Klüver-Barrera, and Prussian blue stains.

For electronmicroscopy, samples of tissues were fixed in 4% glutaraldehyde buffered with cacodylate at pH 7.4 for 4 hr, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol, and embedded in Epon. Thick sections were stained with toluidine blue and thin sections were stained with uranyl acetate and lead citrate.

For comparison, specimens were also obtained from a 10-year-old girl 3 hr after her accidental death.

RESULTS

The presence of homocystine in the urine and blood of this patient was demonstrated on many occasions. Table 1 shows the

Table 1. *Amino acid concentrations*[1]

	Methionine		Cystathionine		Homocystine		Cystine	
	P	C	P	C	P	C	P	C
Plasma[2]	2.39	2.19 ± 0.65[3]	ND	ND	2.97	ND	2.32	2.79 ± 0.98[3]
Urine[4]	17.70	10.38 ± 8.07[3]	ND	4.0[5]	44.67	ND	13.22	21.35 ± 10.51[3]
Frontal lobe[6]	<0.03	0.06–0.15[7]	0.20	0.07–0.55[7]	ND	ND[7]	ND	
Occipital lobe[6]	<0.03	0.14–0.17[7]	0.74	0.08–0.41[7]	ND	ND[7]	ND	
Liver[6]	0.04	0.06–0.54[7]	0.06	0.03–0.13[7]	ND	ND[7]	0.21	

[1] P: patient; C: control; ND: not detected.
[2] Micromoles per dl.
[3] Mean ± SD of 10 control subjects.
[4] Micromoles per 24 hr.
[5] Data from Gerritsen and Waisman (7).
[6] Micromoles per g.
[7] Data from Gerritsen and Waisman (8).

Table 2. *Enzyme activities in tissues*

Subject	Cystathionine-β-synthase[1]	N^5-Methyltetrahydrofolate methyltransferase[2]	5,10-Methylene-tetrahydrofolate reductase[3]
Patient			
Skin fibroblast	20.96	2.17	0.53
Liver	84.80	1.19	0.00
Brain	51.80	1.05	0.59
Control subject			
Skin fibroblast	34.24	2.70	5.14
Liver	189.60	1.83	13.80
Brain	22.00[4]		

[1] Nanomoles of cystathionine formed per 135 min per mg protein.
[2] Nanomoles of methionine formed per hr per mg protein.
[3] Nanomoles of formaldehyde formed per hr per mg protein.
[4] Data from Mudd (21).

concentrations of methionine, cystathionine, homocystine, and cystine in plasma, urine, and tissues.

It is seen that homocystine was detected in the plasma and urine. In the brain and the liver, methionine concentrations were decreased but cystathionine was detected in normal concentrations.

Tissue activity of cystathionine-β-synthase, N^5-methyltetrahydrofolate homocystine methyltransferase, and 5,10-methylenetetrahydrofolate reductase is shown in Table 2. The validity of these enzyme studies in the postmortem tisses was confirmed by enzyme assays with cultured skin fibroblasts showing a deficiency of 5,10-methylenetetrahydrofolate reductase (32).

GROSS PATHOLOGY

The body was that of a fair-haired female, with normal height and body weight. Mild pectus excavatum and genu valgum were present. There was widespread thrombosis involving the superior saggital sinus, lateral sinuses, their tributaries, and the pulmonary artery and its branches. Multiple infarcts were found in the lungs. All the internal organs were of normal size and weight except for the brain which was soft and weighed 940 g (average normal weight: 1,280 g).

LIGHT MICROSCOPY

The liver showed fatty changes, extending from the center of the lobules to the portal triads. Occasionally, fat cysts were observed. There were no Mallory's hyaline bodies. The central veins and sinusoids were dilated and their walls contained increased fibrocollagenous tissues. In the portal triads, there were mild to moderate fibrosis and thickened arterioles with intimal hyperplasia. Bile ductules and portal venules were normal. A moderate increase of iron was present in the hepatocytes.

In the spleen, there was sinusoidal congestion and an increased amount of iron. The sphenic arteries showed hyalinization and contained prominent endothelial cells.

Multiple areas of recent infarction were found in the lungs. Recent thrombi were observed in the pulmonary arterioles and capillaries.

The thymus contained a well preserved lymphoid population but epithelial cells were scanty and Hassall's corpuscles were indistinct.

Focal lesions were observed in the aortic wall. There was a moderate degree of intimal hyperplasia associated with regional areas showing sloughing of endothelium. The media showed focal fragmentation and disruption of the elastic lamellae (Fig. 1), the adventitia showed minimal fibrosis, and the vasa vasorum penetrated deeply into the media of the aorta. Focal fragmentation of the elastic lamellae was also found in the iliac and mesenteric arteries. Arterioles in the brain and skeletal muscles showed thickened walls with fibrosis and intimal hyperplasia with swollen endothelial cells.

In the cerebral cortex the major abnormality was in the white matter, consisting of patchy, incomplete perivascular and confluent demyelination with moderate astrocytosis (Fig. 2). There was a paucity of microglial elements. The oligodendrocytes were unremarkable. Cerebral vessels in the deep cortex showed prominent endothelial cells and thick hyalinized walls (Fig. 3), sometimes resembling fibrinoid necrosis. A few of the vessels were thrombosed and were surrounded by a widened perivascular space. Demyelination was out of proportion to the vascular changes. Surprisingly, the vessels in areas where myelination was preserved were cuffed by microglial cells and lymphocytes, whereas demyelinated areas contained vessels devoid of microglial elements. The compound granular corpuscles were found in small numbers. In the gray matter, there were ischemic changes in the form of neuronal loss in the second and third layers with focal areas showing shrunken cells and basophilia. The thalamus and globus

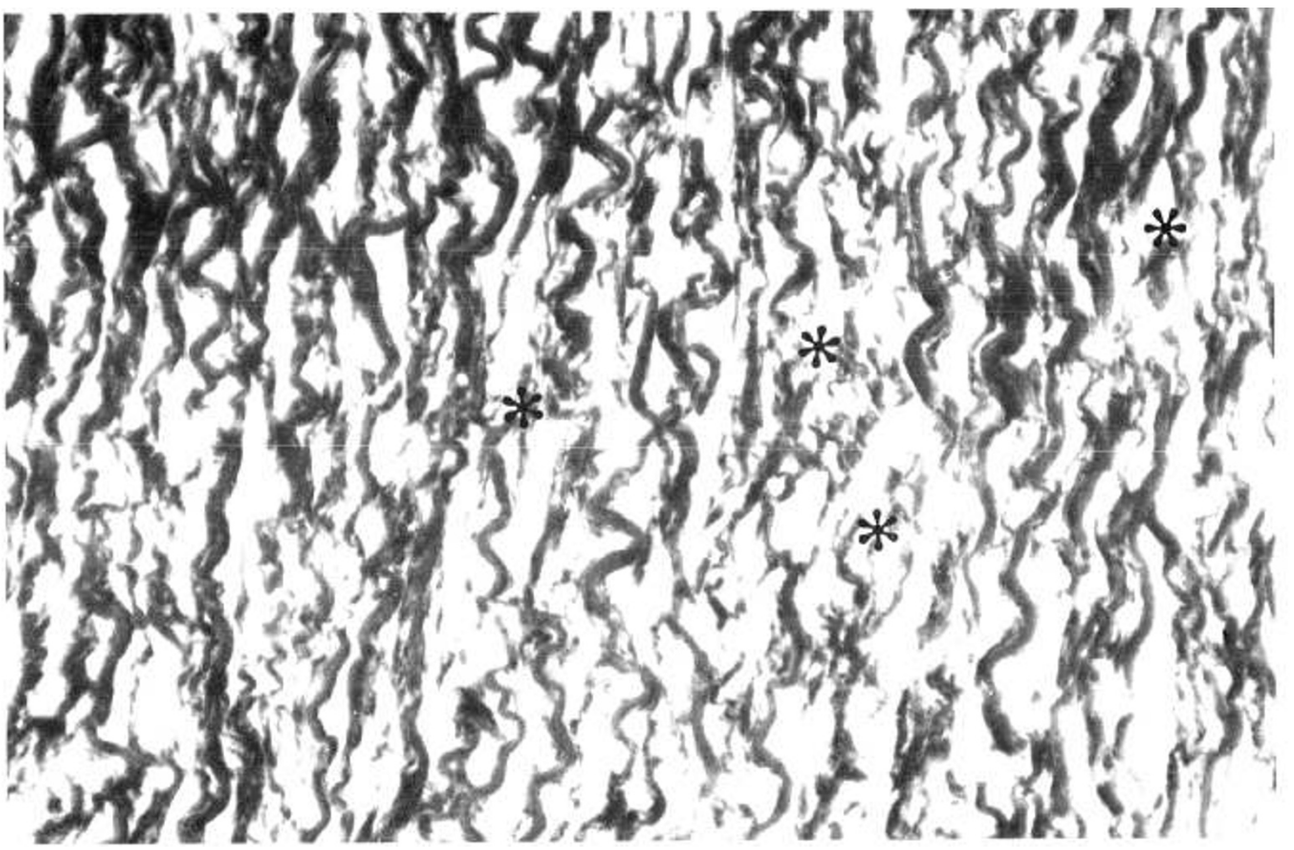

Fig. 1. Photomicrograph of thoracic aorta (× 320; elastica) showing focal fragmentation of elastic lamellae (*).

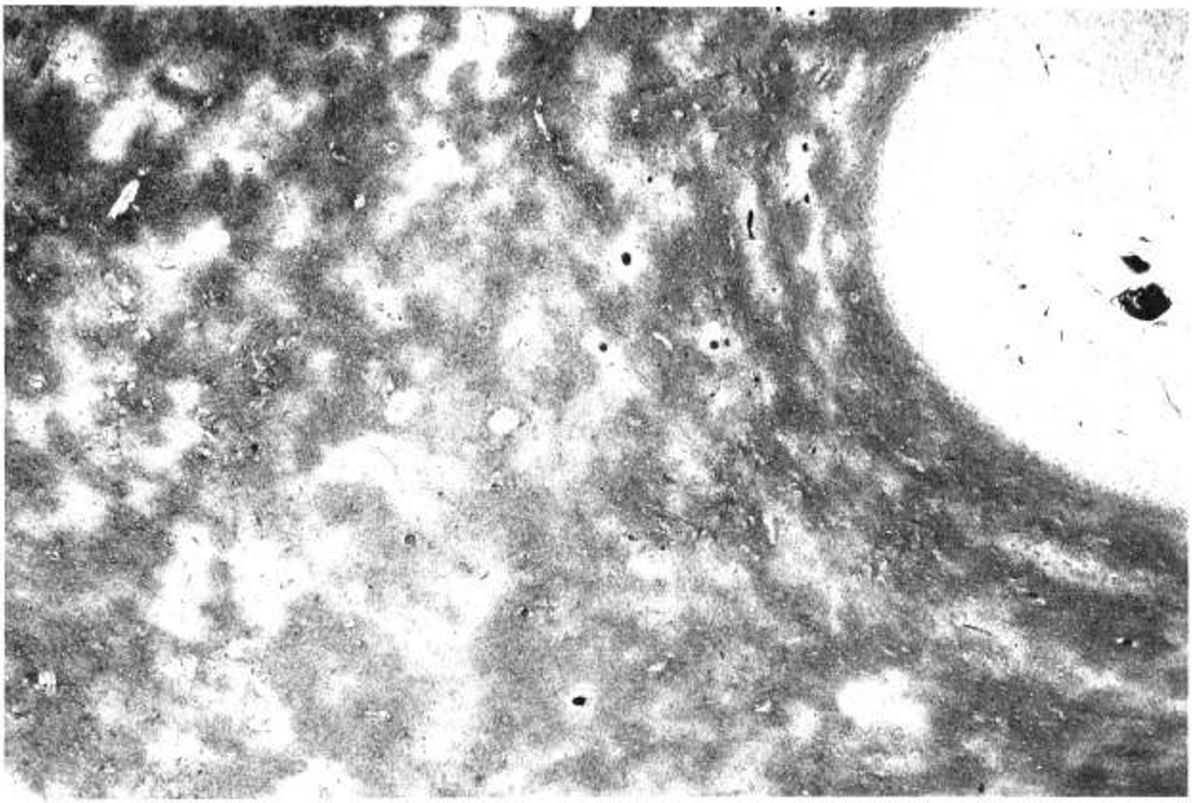

Fig. 2. Cerebral cortex (× 20; Weil) showing multiple foci of demyelination and thrombi in blood vessels.

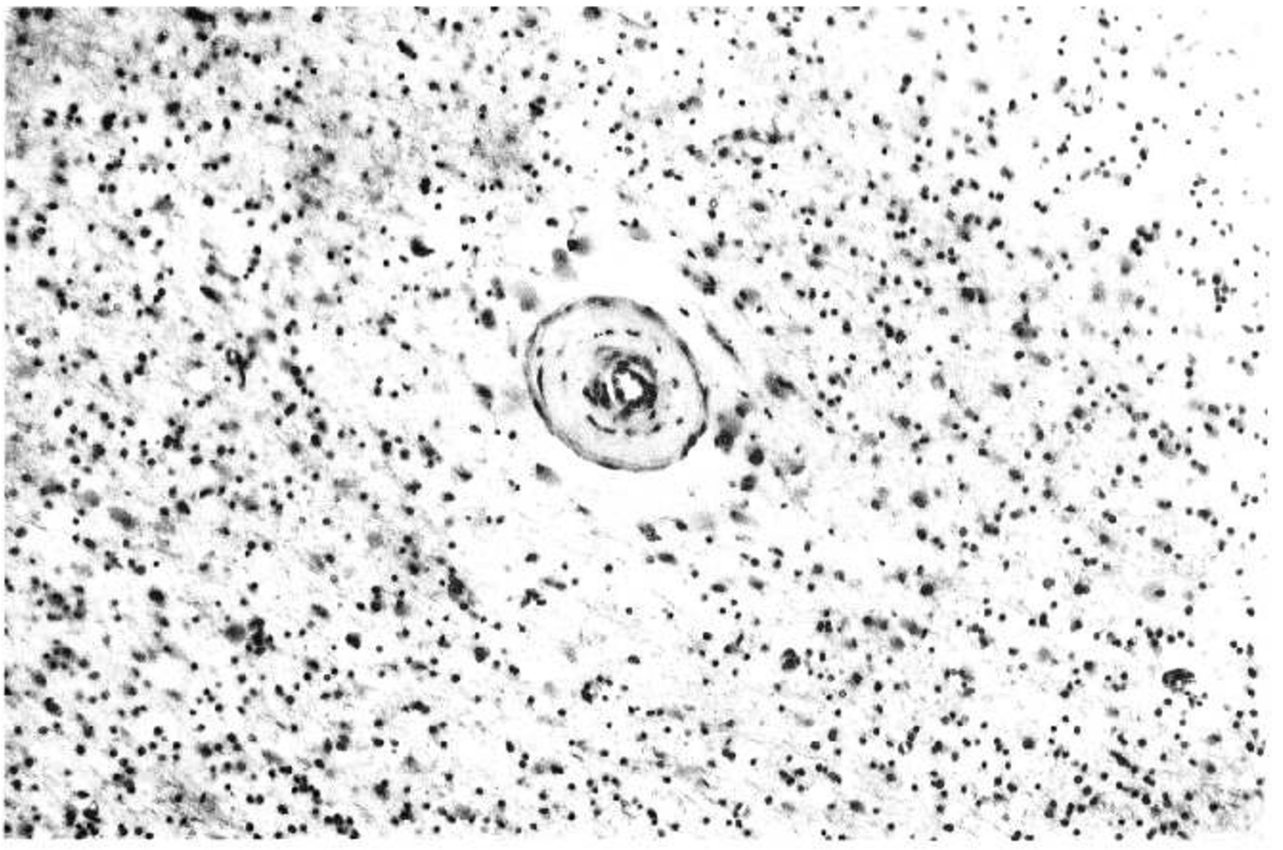

Fig. 3. Photomicrograph of deep cortex (× 200; Klüver) showing an arteriole with hyalinized wall and narrow lumen, enlargement of the perivascular space and the surrounding astrocytes.

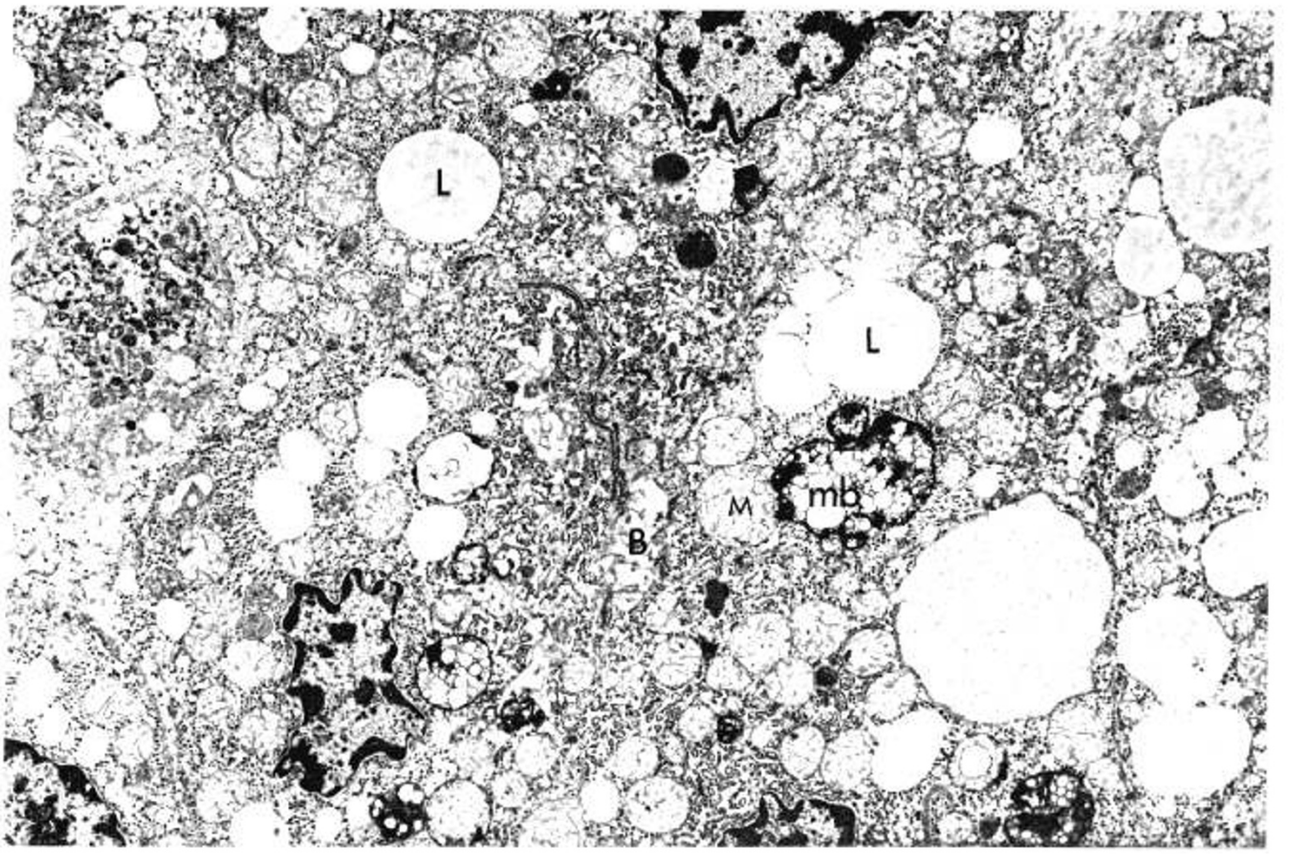

Fig. 4. Electron micrograph of hepatocytes (× 6,500) showing an increased amount of lipid bodies (*L*), multivesicular bodies (*mb*), glycogen, rough endoplasmic reticulum, mitochondria (*M*), and bile canaliculus (*B*).

pallidus were normal but the pons and medulla showed focal demyelination in the corticospinal tracts with minimal gliosis. In the cerebellum the deep white matter showed similar although less marked changes than in the cerebral cortex.

ELECTRON MICROSCOPY

In the liver (Fig. 4), the borders of the hepatocytes were well demarcated. Numerous low density lipid droplets measuring 2–5 μm in diameter were observed. Peculiar membrane-bound multivesicular bodies with variable matrical density material measuring 0.1–0.4 μm in diameter were found. These bodies were close to the lysosomes and were seen within the phagosomes. In other areas, electron-dense bodies resembling ferritin or lipofuchsin pigments were also seen. A few mitochondria showed finger-like projections, whereas others were bizarrely shaped. Giant mitochondria were also observed, but their cristae per unit size were approximately normal (Fig. 5). Numerous myelin figures were seen in the vicinity of degenerating mitochondria and liposomes (Fig. 6). Rough endoplasmic reticulum and glycogen granules were found in abundance. The smooth endoplasmic reticulum was not prominent. There were normal amounts of lysosomes and the nuclei were normal. Focal increase of pericellular collagen was observed. The space of Disse contained swollen microvilli, occasional mitochondria, multivesicular bodies, and lipid droplets. The walls of the central veins showed marked increase in collagen. Mild reactive changes in Kupffer cells were observed. Bile canaliculi were normal.

In the aorta, mild hypertrophy of the remaining endothelial cells was observed. The subendothelial space was increased and was occupied by an increased amount of collagen. The media showed fragmentation and disruption of the elastic lamellae which contained electron-dense reticulate fibers (Fig. 7). The interlamellar space was increased and was occupied by collagen and moderately electron-dense amorphous material. Smooth muscle cells in the media were shrunken and were irregularly formed. Only peripheral attachment devices or intracytoplasmic myofilamentous condensations were demonstrable. In contrast to the smooth muscles of normal aorta, individual myofilaments were seen with difficulty. The abnormal smooth muscle cells showed a slight increase in electron density. Glycogen granules and rough endoplasmic reticulum were slightly reduced. Nuclei were indented but were otherwise normal. Similar changes were observed in samples obtained from the iliac and mesenteric arteries.

Skeletal muscles showed focal fragmentation, disruption, and smearing of the Z discs (Fig. 8). Oblong rods measuring 0.1–0.2 μm by 0.2–0.4 μm were found. In areas where Z disc changes were observed, myofilaments and the A, H, and I bands were disorganized. Lysosomes, glycogen, nuclei, and sarcolemma were normal. The endothelial cells of muscular arterioles showed minimal injury in the form of dilation and hypertrophy of the endoplasmic reticulum and an increase of ribosomes and lysosomal bodies, as well as swelling of the mitochondria. Myelin figures were also found. The nuclei were normal. The basal lamina showed an increase of collagen but the lining smooth muscle cells were mostly normal.

In the brain, the changes observed in the neurons of the cerebral cortex and in the Purkinje cells of the cerebellar cortex were similar. Nissl bodies were disorganized; the rough endoplasmic reticular cisterns were in disarray; and ribosomes and glycogen granules were markedly increased (Fig. 9). Occasional intracytoplasmic filamentous or crystalline and Hirano bodies were found in the Purkinje cells and cortical neurons, respectively (Figs. 9, 10). In addition, lamellar bodies and relatively electron-dense proteinaceous deposits in the vicinity of cisterns of the Purkinje cells were observed (Fig. 11). The macroglial elements (astrocytes and oligodendrocytes) showed reactive changes in the form of increased intracytoplasmic fibrils, glycogen particles, and electron-dense bodies. Their cisterns were also in disarray. The neuropile of the cortex, pons, and medulla showed striking and similar changes.

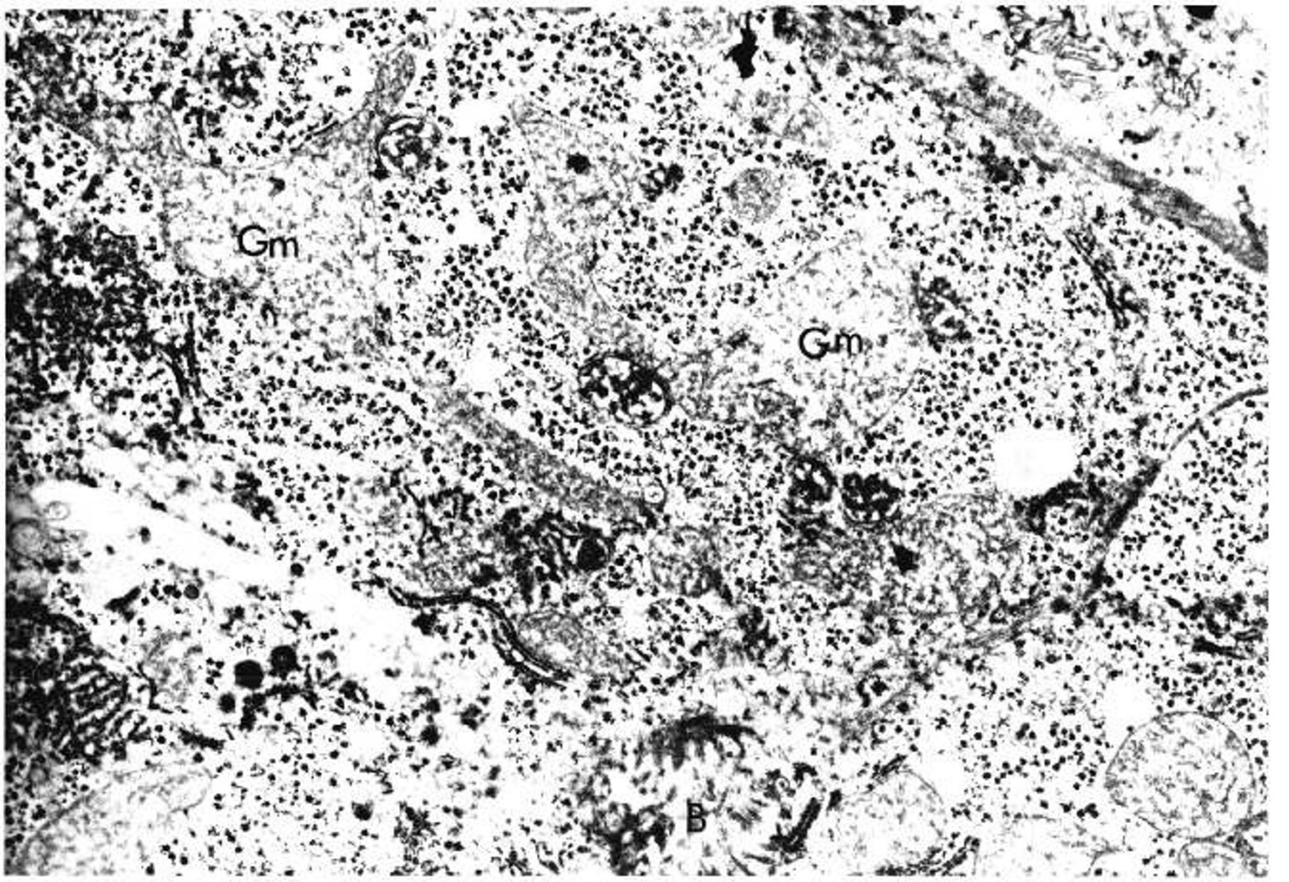

Fig. 5. Electron micrograph of hepatocytes (× 10,000) showing bizarre, giant mitochondria (*Gm*) and bile canaliculus (*B*).

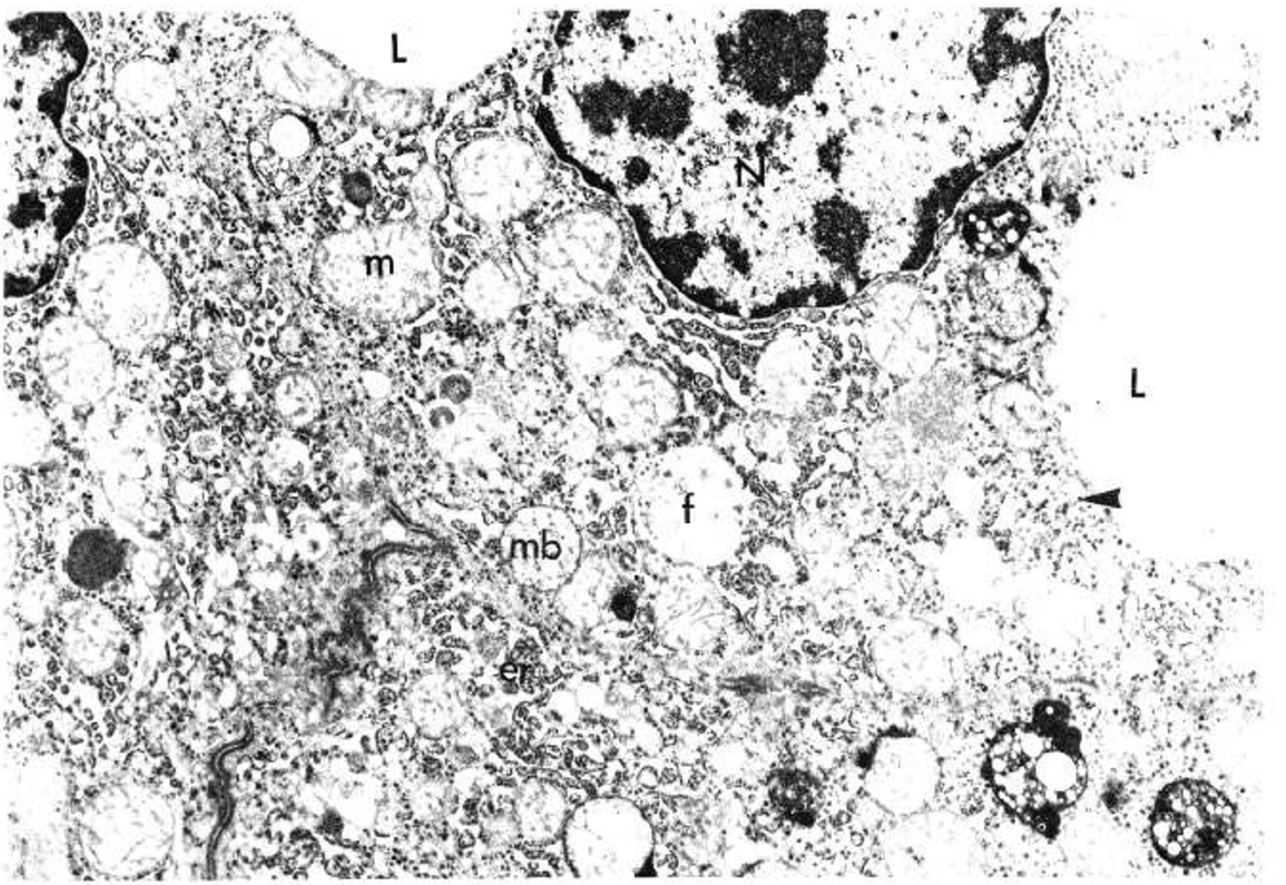

Fig. 6. Electron micrograph of hepatocytes (× 10,500) showing an increased amount of multivesicular bodies (*mb*), myelin figures (*f*), lipid bodies (*L*), dilated rough endoplasmic reticulum (*er*), glycogen (*arrow*), mitochondria (*m*), and nucleus (*N*).

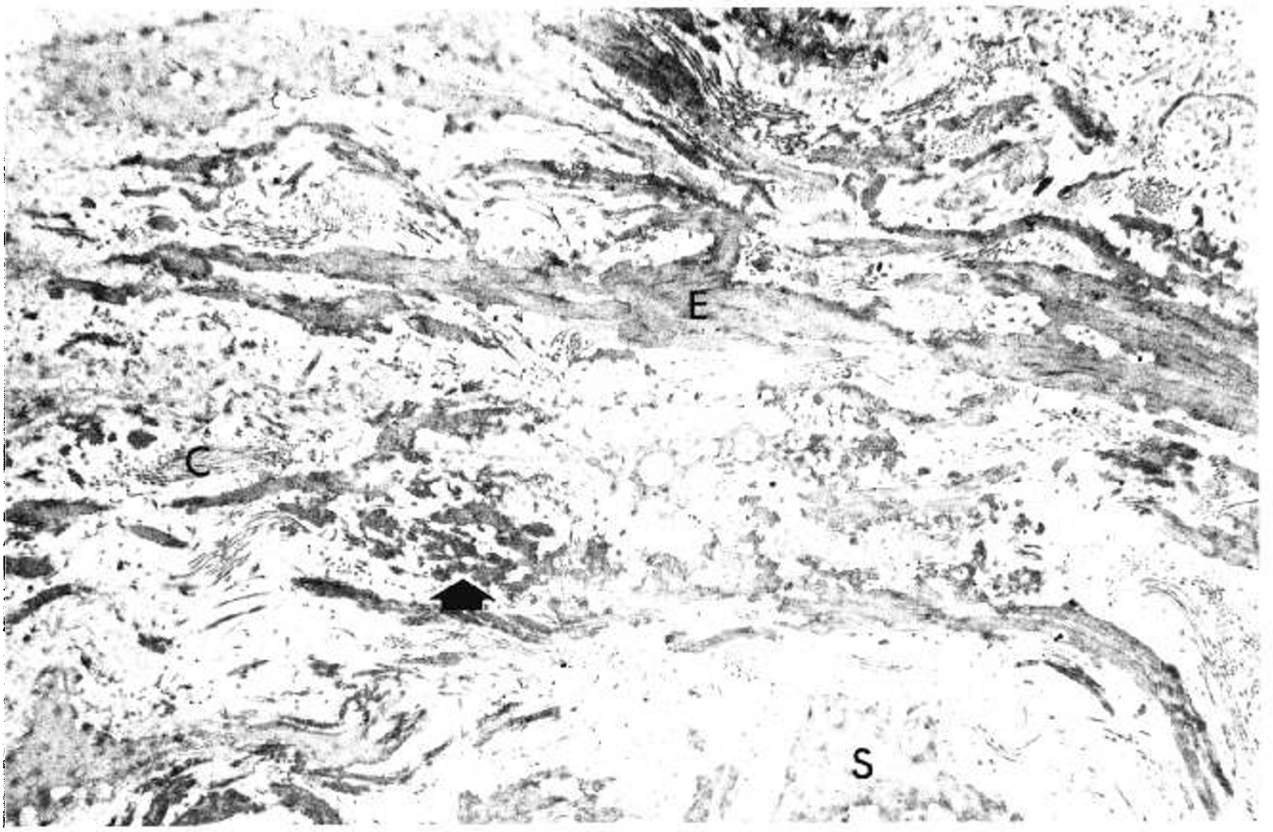

Fig. 7. Electron micrograph of medial portion of the thoracic aorta (× 6,500) showing thin and fragmented elastic lamellae (*E*), irregularly formed smooth muscle (*S*), electron-dense amorphous deposits (*arrow*) in the loose interlamellar space, and collagen (*C*).

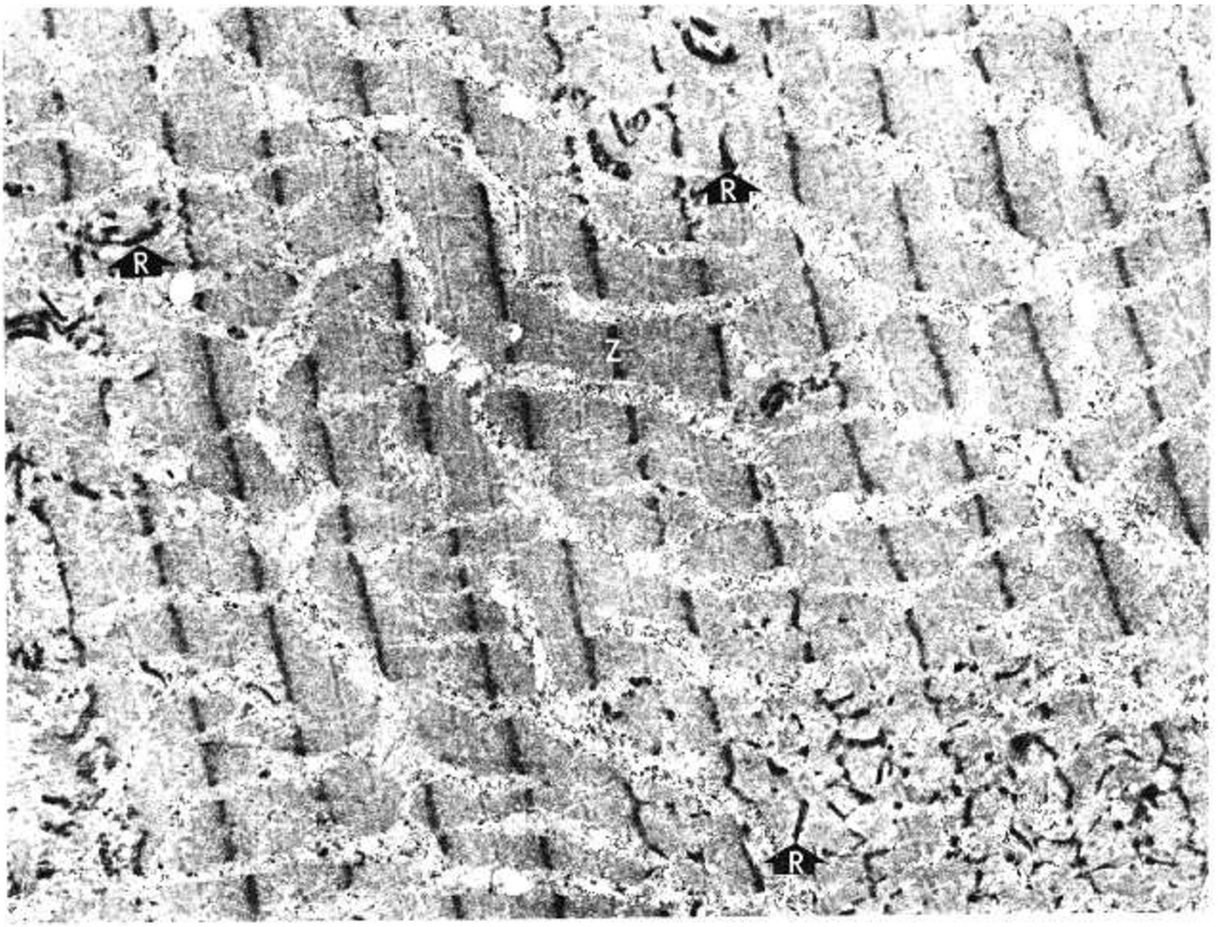

Fig. 8. Electron micrograph of skeletal muscle (× 7,500) showing focal areas of fragmentation and disruption in the Z lines (*Z*) and rod (*R*) formation.

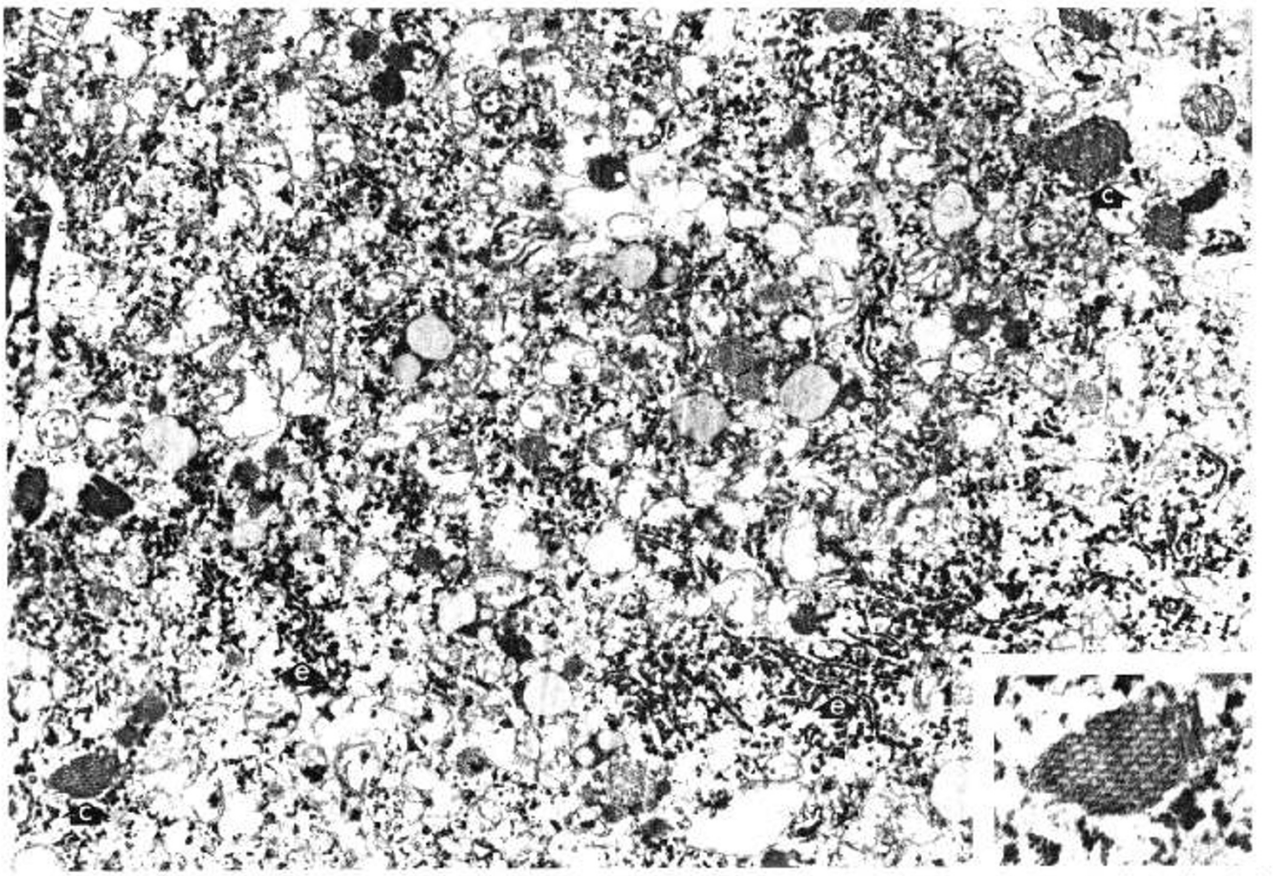

Fig. 9. Electron micrograph of a Purkinje cell in the cerebellar cortex (× 10,500) showing the disarray of the rough cisternal endoplasmic reticulum (*e*), crystalline bodies (*c*), and a relative increase of ribosomes and glycogen. *Inset* showing a crystalline body at a higher magnification.

Fig. 10. Electron micrograph of cerebral cortex (× 9,050) showing a neuron, synaptic buttons (*arrowheads*), Hirano body (*H*), and nucleus (*N*).

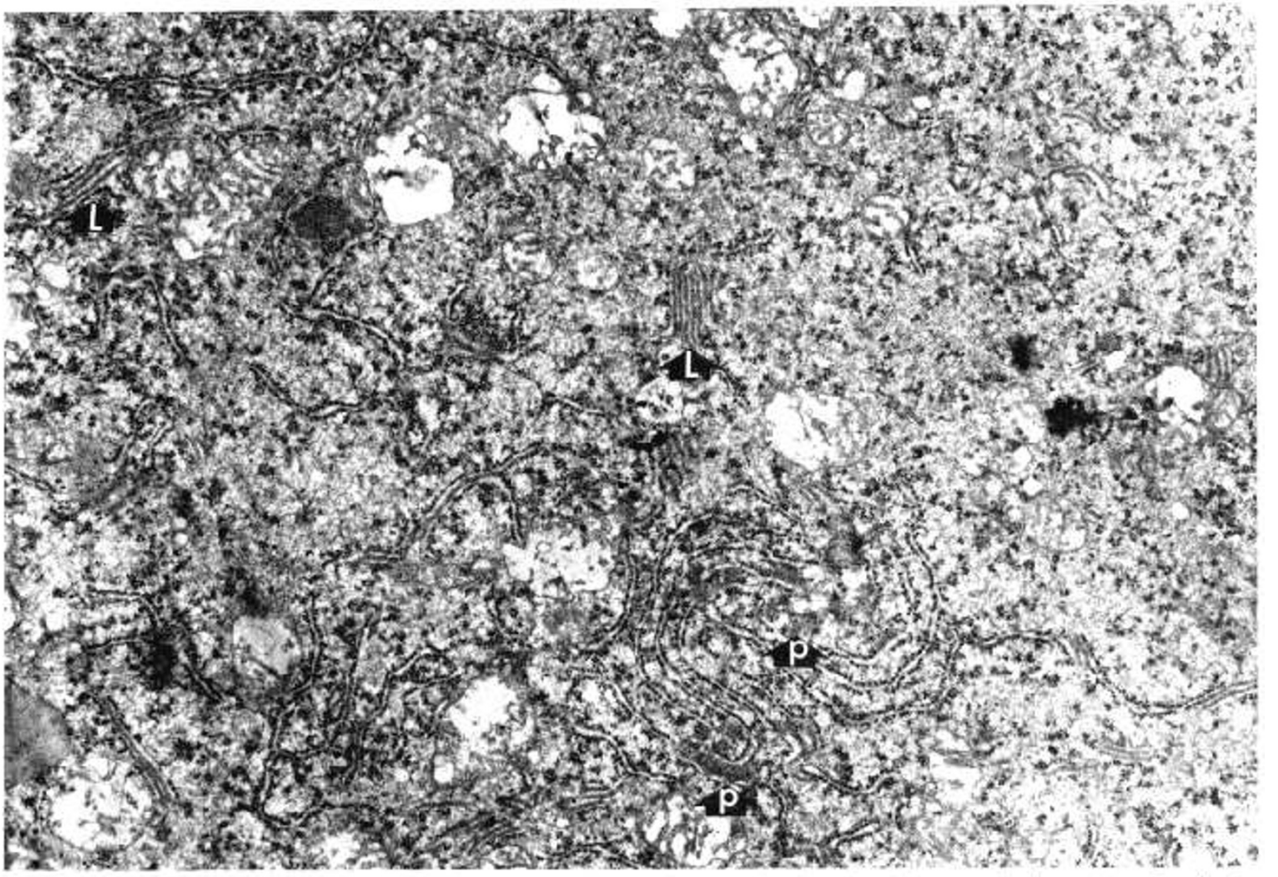

Fig. 11. Electron micrograph of a Purkinje cell (× 12,500) showing amorphous proteinaceous deposits between the cisterns (*p*) and lamellar bodies (*L*).

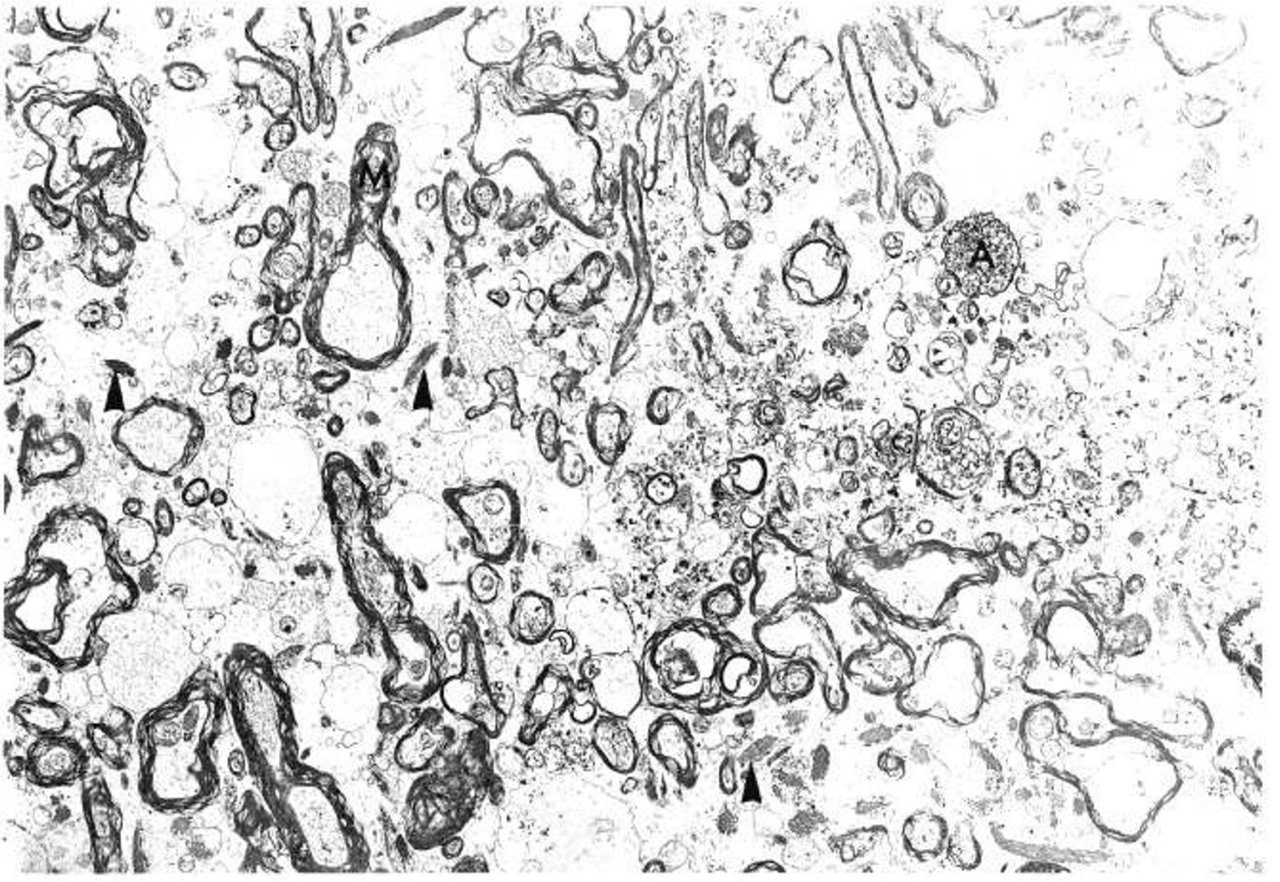

Fig. 12. Electron micrograph of neuropile of cortex (× 4,000) showing demyelination of myelin sheath (*M*), filamentous bodies (*arrowheads*), vesiculated bodies in completely demyelinated axon (*A*), and a paucity of synaptic buttons.

There were areas of moderate demyelination with focal wrinkling and dissolution of the periodicity of the myelin sheath (Fig. 12). Some of the demyelinated axons contained only a thin rim of myelin sheath. In some areas, the axoplasm was displaced outwards and the periaxonal space was swollen (Fig. 13). In these axons, there was an increase of electron density and focal disruption of the neurotubules. Some areas, especially in the medulla, where complete disappearance of axons occurred, were filled with vesiculated bodies, degenerated mitochondria, and myelin figures. Numerous filamentous bodies measuring 0.2 0.7 μm in size were observed. In the neuropile of the cortex, there were areas where synaptic buttons were few whereas, in other areas, they were found in normal numbers (Figs. 10, 12).

The kidneys showed minimal abnormalities. The glomeruli were generally shrunken with partially compressed capillary spaces. The basement membranes showed mild but diffuse thickening of the lamina densa; and the lamina rarae were indistinct. Wrinkled basement membranes were found in many areas, especially those near the mesangial zones. Wrinkling was not associated with splitting. There was a slight increase of the mesangial matrix. Epithelial cells and endothelial cells were swollen and the epithelial cells showed prominent primary trabecular processes which attached directly to the basement membranes more frequently than usual.

DISCUSSION

Morphologic (1, 2, 9) and electron microscopic studies (6, 18, 25, 27) in patients with cystathionine-β-synthase deficiency have been reported by various authors. In addition, morphologic studies in patients with N^5-methyltetrahydrofolate homocystine methyltransferase deficiency have also been described (18). However, this communication records the first detailed morphologic and electron microscopic description in a patient with 5,10-methylenetetrahydrofolate reductase deficiency. The lesions observed in the latter share many common features with patients suffering from other forms of homocystinuria, suggesting the presence of a common factor in the pathogenesis of many of the structural abnormalities.

The predominant abnormalities were found in the arterial bed. Widespread vascular injury and thrombosis were observed in our patient as in patients with other types of homocystinuria. It is of interest that similar lesions have been observed in a metabolic defect of vitamin B_{12} (3). The latter is converted to methyl-B_{12}, the cofactor of N^5-methyltetrahydrofolate homocystine methyltransferase.

Two hypotheses have been postulated to account for the thrombotic tendency in patients with homocystinuria. In 1964, McDonald *et al.* (20) suggested that homocystine and other sulfur-containing amino acids, such as methionine, caused increased "platelet stickiness," and that this might be the cause of many vascular accidents in homocytinuric patients. However, other investigators were unable to substantiate their findings (5), and platelet function studies in our patient revealed no abnormality (32). In addition, the changes in the vascular wall observed in patients with homocystinuria are not generally found in vessels with recent or old thrombi secondary to hypercoagulability of the serum or platelet dysfunction.

A second hypothesis to account for the thrombotic tendency is the occurrence of an initial vascular injury. In our patient, as well as in patients with other forms of homocystinuria, widespread endothelial and subendothelial changes, involving the smooth muscles and elastic tissues, were observed. These changes often occurred without thrombosis, suggesting that any thrombosis is secondary to the preceding vascular lesions. It seems that homo-

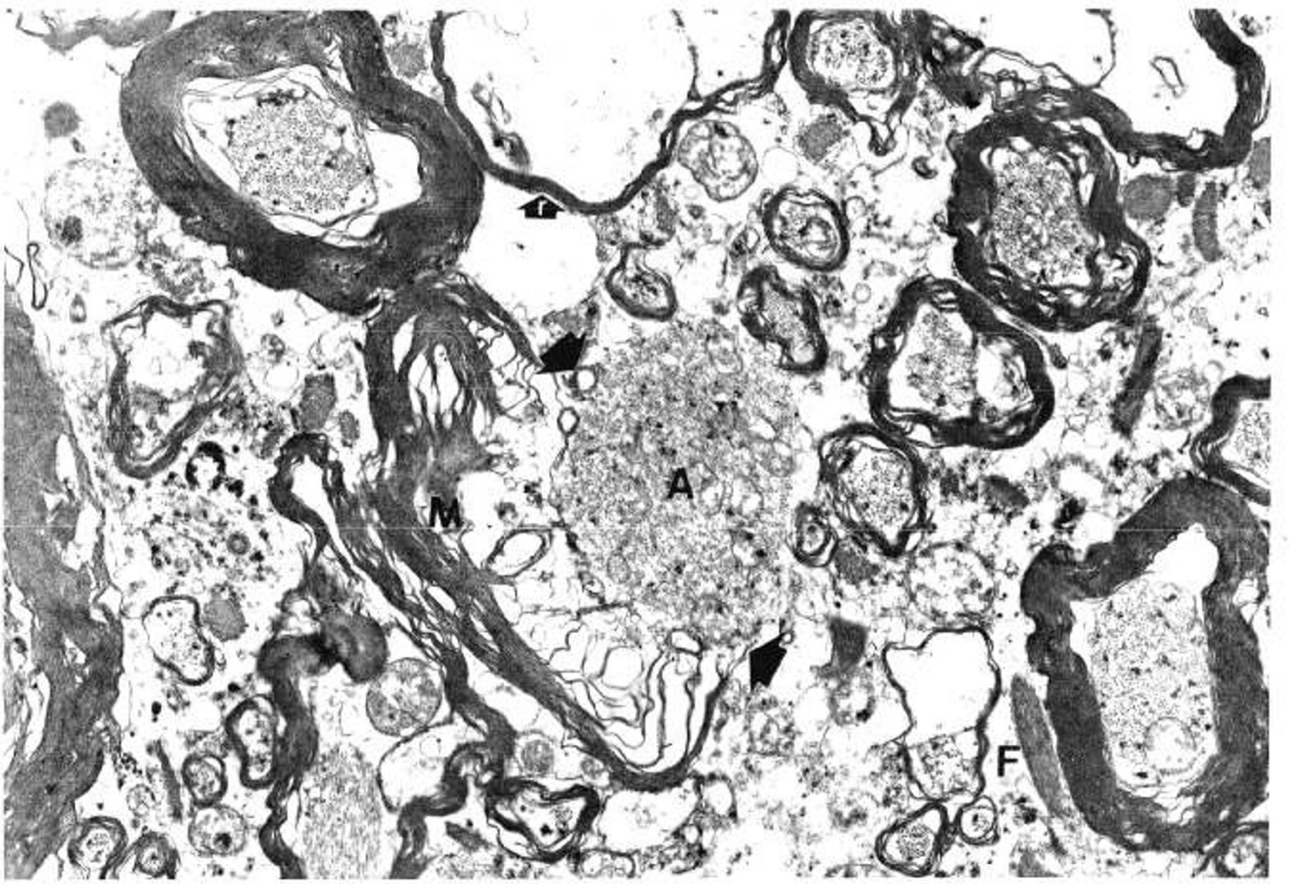

Fig. 13. Electron micrograph of medulla (× 10,500) showing demyelinated axon (*A*) completely displaced out of the myelin sheath (*large arrows*) and enlargement of periaxonal space, wrinkling and loss of periodicity of myelin sheath (*M*), the thin rim of a remaining myelin sheath (*r*), and filamentous body (*F*).

cystine is the most likely "toxic agent" to the vasculature, as it is detected in the plasma in all forms of homocystinuria and as methionine is normal or subnormal in deficiency of N^5-methyltetrahydrofolate homocystine methyltransferase and 5,10-methylenetetrahydrofolate reductase. The observations in experimental homocystinuria in baboons (11) provide further support to this hypothesis. However, it is not known if the initial injury is in the endothelium, resulting in subsequent damages in the elastic tissues and smooth muscles, or if the initial injury is in the connective tissues, leading to subsequent changes in the endothelium. The observations that the collagen obtained from homocystinuric patients (12) and that proteoglycans synthesized by skin fibroblasts from these patients (19) were abnormal tend to support the latter possibility.

The thrombosis in the dural sinuses and cerebral veins was neither accompanied by cerebral infarction nor significant changes in the vessel wall, suggesting a recent and terminal event. The reason for the lack of damage in the venous walls is unexplained.

The conspicuous demyelination observed by light as well as by electron microscopy and the disorganization of cisternal array in the cells of the central nervous system may be due to ischemia and thrombosis secondary to the vascular disease. However, the demyelination, which is perivascular to some extent, is out of proportion to the vascular lesions, thus suggesting the effect of a diffusible toxic agent. The presence of crystalline and lamellar bodies in the Purkinje cells and Hirano bodies in the cortical neurons possibly reflects disordered protein metabolism of the cells. These have been seen in amyotrophic lateral sclerosis, Parkinsonism-dementia complex, and Pick's disease (13, 15, 26). The presence of minimal gliosis also suggests a sustained toxic injury rather than the sole responsibility of the vascular lesions. It seems, therefore, that ischemia and vascular thrombosis are not the only causative factors in the structural abnormalities in the brain.

The nature of the abnormalities in the hepatocytes remains speculative. Giant mitochondria, abnormal changes in the rough endoplasmic reticulum, myelin figures, and excessive lipid droplets can be induced by chemical agents such as alcohol, phenobarbital, and chlorpromazine (29). Megamitochondria have also been observed in obstructive jaundice, Rotor syndrome, and riboflavin deficiency (28). Luck has shown that in choline-dependent neurospora, deprivation of choline results in enlargement of the mitochondria (17). It may be suggested that deficiency of methyl donors such as choline, methionine, or methyltetrahydrofolate may be responsible for the abnormalities in the mitochondria and the fatty changes. However, similar changes are observed in cystathionine-β-synthase deficiency without a lack of methyl donors. The multivesicular bodies, similar to those observed in Wilson's disease and alcoholic cirrhosis (29), and the autophagic vacuoles suggest a rapid turnover of the organelle. The remarkably dilated central veins and increase of perivascular collagen may be due to intrahepatic congestion secondary to microthrombi formation. In contrast to Gaull and Schaffner's observations on patients with cystathionine-β-synthase deficiency (6), we did not detect any increase in smooth endoplasmic reticulum in the hepatocytes, thus supporting their contention that the increase in smooth endoplasmic reticulum was probably an expression of high tissue concentrations of methionine.

Fragmentation, disruption, and smearing of the Z discs in skeletal muscles have been observed in various disorders such as myotonic dystrophy, cold injury, and alcoholism (24). These changes in the Z discs and the rod formation are probably nonspecific or ischemic in nature.

The renal abnormalities, however, can best be explained on the basis of renal ischemia.

In conclusion, the similarities of the pathologic abnormalities in this patient to other types of homocystinuria suggest that homocystine is the agent responsible for the widespread lesions in the vasculature, brain, and liver. In addition, ischemia secondary to vascular disease plays a role in some of the pathologic changes, especially in the skeletal muscle and kidney.

SUMMARY

Morphologic studies on a patient with homocystinuria due to 5,10-methylenetetrahydrofolate reductase are described.

The arterial lesions were prominent and showed fragmentation of the elastic lamellae. Perivascular demyelination and thrombosis were observed in the brain. Hirano bodies in the cortical neurons and crystalline and lamellar bodies in the Purkinje cells were present. Lipid droplets, giant mitochondria, myelin figures, and membrane-bound multivesicular bodies in the hepatocytes were seen. Fragmentation of the Z discs of striated muscle and wrinkling of the glomerular basement membrane were observed.

The morphologic lesions observed in this patient were, to some extent, similar to those described in patients with homocystinuria with either cystathionine-β-synthase or N^5-methyltetrahydrofolate homocystine methyltransferase deficiency. Furthermore, it is suggested that the pathologic changes observed are probably "vasculotoxic" in nature.

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Calcium
cystic fibrosis
mucin

Effects of Calcium on Intestinal Mucin: Implications for Cystic Fibrosis

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Extract

A major feature of the disease cystic fibrosis is the excessive concentration of mucus within ducts and glands of mucous-producing organs. Some mucous secretions also show an elevation in calcium concentration. Using purified rat intestinal goblet cell mucin as a model mucin, we have investigated the effect of millimolar additions (1–25 mM) of $CaCl_2$ on the physical properties of the mucin. Isotonicity of incubation media was preserved in order to mimic *in vivo* conditions. $CaCl_2$ (8–15 mM) caused a 15–33% decrease in viscosity, no change in electrophoretic mobility in acrylamide gels, and a 20–30% decrease in solubility of the mucin. Solubility changes were reversed by the addition of EDTA (20 mM) to incubations. Insolubility was also produced in incubations of mucin with a mixture of soluble intestinal contents (NaCl washings). These findings strongly suggest that the mucin became smaller and more dense as calcium was added, a process most probably achieved by loss of intramolecular water.

Speculation

It is hypothesized that elevated concentrations of calcium within glycoprotein secretions of patient with cystic fibrosis may substantially increase the density and insolubility of mucins, promoting the formation of mucous "plugs."

Mucous secretions of patients with cystic fibrosis are excessive in amount in salivary glands, bronchi, intestine, gall bladder, and cervix (7, 12, 14). The calcium concentration in many of these secretions is also high, ranging from "slightly elevated" to over 100-fold in excess of normal (12). It has been hypothesized that Ca may contribute to the formation of mucous "plugs" in glands and ducts by causing polymerization, aggregation, or gel formation of otherwise normal mucin macromolecules. A few studies have shown that Ca is responsible for aggregation of small salivary proteins (4, 5, 13), but no studies have been performed to discover whether Ca changes the physical properties of purified mucin macromolecules of intestinal or bronchial origin.

Over the past few years a native goblet cell mucin has been purified in our laboratory from rat small intestine (9, 11). It resembles mucins purified by others from several human and animal organs (1, 4, 6, 17–19), in that it is a polydisperse viscous glycoprotein rich in carbohydrate, containing high quantities of serine, threonine, and proline within the peptide core.

We have used rat goblet cell mucin as a model for the investigation of Ca-mucin interaction. Previously we showed that rat mucin binds Ca ions, especially under conditions of low ionic strength, but that during binding Ca caused only minor physical changes in the mucin (8). In the present study we have measured the effects of Ca upon some of the physical properties of mucin in isotonic media in order to mimic more closely *in vivo* conditions. Our results indicate that Ca may alter the three-dimensional architecture of mucin so as to increase its density and exclude water. It is postulated that this mechanism may be involved in the formation of mucous plugs in cystic fibrosis.

METHODS

The preparation of goblet cell mucin (GCM) from rat intestine, radioactive labeling of the mucin using precursor [$^{14}C_1$]glucosamine, the techniques of polyacrylamide disc gel electrophoresis, analytic ultracentrifugation, and measurement of solubility of the mucin have been described in detail in earlier publications (8, 9, 11). Minor modifications and specific details are included as appropriate under *Results*.

Intestinal washings, when required for solubility experiments, were collected by washing the entire small intestine of each of 10 rats with approximately 20 ml 0.15 M NaCl. The washings were pooled, centrifuged at 4° at 30,000 × *g* for 15 min to remove particulate material, and the suernatant solutions saved. The soluble supernatant fluid was stored frozen in a dilute state or else concentrated isotonically 10-fold by an Amicon Dia-Flo ultrafilter