HURLER'S SYNDROME

A Histochemical Study. New Techniques for Localization of Very Water-Soluble Acid Mucopolysaccharides

Hubert J. Wolfe, M.D.; John B. Blennerhasset, M. B.; Gilbert F. Young, M.D., and Richard B. Cohen, M.D.

From the Department of Pathology, Harvard Medical School; The James Homer Wright Pathology Laboratories, Charles S. Kubick Laboratory for Neuropathology and The Joseph P. Kennedy, Jr. Memorial Laboratories of the Massachusetts General Hospital.

The basic defect responsible for Hurler's syndrome has yet to be elucidated. Much of the data reported has been either divergent or contradictory. It is especially difficult to compare the results of histochemical studies from case to case since methods of tissue fixation, histochemical techniques, and organs examined have varied considerably. To further complicate comparisons the clinical severity of Hurler's syndrome varies and *formes frustes* are recognized.[1] We wish to present here the histochemical analysis of two cases of Hurler's syndrome including a discussion of the application of recently developed techniques for localization of extremely water-soluble acid mucopolysaccharides.[2] The use of these methods clarifies aspects of the tissue alterations and of the biochemical nature of the cellular inclusions, heretofore obscure because of the inadequacy of available methods for their proper histochemical identification.

Hurler's syndrome was recognized as a clinical entity in the early part of the 20th century.[3,4] Some investigators speculated that the disease might represent a form of lipoidosis,[5] particularly since a stainable lipid was noted to accumulate in the central nervous system[6] and other organs. Washington suggested the term lipochondrodystrophy to replace the eponym, Hurler's syndrome.[7] More recently, however, workers have felt the defect to be a disorder of polysaccharide metabolism. On the basis of histochemical studies in three cases, Lindsay and co-workers concluded that the accumulated material represented a glycogen.[8] In 1952, Brante,[9] using histochemical and biochemical methods, reported the accumulation of sulfated mucopolysaccharide in the viscera. Following this observation, abnormal urinary excretion of chondroitin sulfate B and heparitin sulfate was reported.[10,11] Biochemical analyses of

This research was supported in part by Grant No. C-3424 of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and by United States Public Health Service Training Grant in Pathology, No. 2G-192.

Accepted for publication, July 2, 1964.

liver, spleen and other organs have demonstrated the abnormal accumulations of these two acid mucopolysaccharides (AMP).[12] Despite the biochemical evidence demonstrating the storage of AMP, efforts to localize it histochemically have generally been unsatisfactory. The number of different histochemical methods applied by various workers attest to the difficulty involved in the localization and identification of the AMP deposits.[2,8,9,13,14]

Although Hurler's syndrome is now generally classified by most workers as a mucopolysaccharidosis,[15] the accumulation of the lipid component has been repeatedly noted both in histochemical and biochemical studies.[9,16,17] Seitelberger demonstrated a glycolipid in both the central nervous system and visceral organs and on the basis of his findings suggested Hurler's syndrome be classified as a sphingolipoidosis.[18]

Material and Methods

Formalin fixed tissues were processed in the usual manner, paraffin embedded, cut at 6 μ and stained with hematoxylin and eosin (H & E).

Polysaccharide Studies

Fresh blocks, 4 mm in thickness, were fixed in dioxane for 24 hours and paraffin embedded or double embedded in celloidin and paraffin. The paraffin embedded tissues were cut at 6 μ and transferred to a 10 per cent solution of cetyltrimethylammonium bromide in 10 per cent formalin for 30 to 90 minutes prior to contact with watery solutions. Sections treated in this manner were then stained with 1 per cent aqueous toluidine blue, alcian blue, periodic acid-Schiff (PAS) alcian blue and dialysed iron. The double embedded material was processed without any contact with watery solutions and the 6 μ sections were stained in a series of 1 per cent toluidine blue solutions in 80, 70 and 50 per cent ethyl alcohol, differentiated and dehydrated in absolute acetone, cleared in xylene and mounted. The use of these techniques has been previously described.[2] PAS and alcoholic PAS reactions were also performed on the double embedded materials. Extractions performed on 6 μ sections of paraffin and double embedded dioxane fixed tissues prior to staining included distilled water at room temperature for 5 minutes, chloroform methanol (2:1) at 50° C for 24 hours and pyridine at 60° C for 24 hours.

Lipid Studies

For the study of lipids fresh tissue was frozen on dry ice and cut at 10 μ in a cryostat at −18° C and placed on slides to dry. The following histochemical stains were employed; 1 per cent aqueous toluidine blue, PAS, PAS orange II, methyl green pyronin, performic acid-Schiff (PFAS), alcian blue, methylene blue extinction reaction, Baker's reaction for phospholipids, Sudan black B, Sudan red and Bial reaction.[19,20] PAS was performed following diastase digestion with saline controls and following acetylation. Extractions were performed on the fresh frozen sections with distilled water at room temperatures, 60° C and 90° C for one hour, with chloroform methanol (2:1) at 50° C for 24 hours and with pyridine 60° C for 24 hours.

CLINICAL HISTORIES

Case 1. W. J. was born of normal parents. There were 5 siblings, all male. One died of pneumonia at 1 month of age. Another brother had the typical stigmas of

gargoylism. W. J. was apparently normal at birth but his development was slow. By age 4 a marked degree of mental retardation was obvious. When he was 9 years old, the patient was admitted to Paul A. Dever State School where the typical appearance of Hurler's syndrome was noted. At that time no cardiac murmurs were heard, but the abdomen was protuberant with moderate hepatosplenomegaly and a large umbilical hernia. His estimated IQ was recorded as 11. Significant laboratory findings included high concentration (100 mgm per 100 ml) of urinary acid mucopolysaccharide and some leukocytes contained "toxic granulations." During the subsequent 4 years he grew very little in height and weight and his behavior showed little change. He suffered from occasional upper and lower respiratory tract infections and died at age 13 after a severe, lower respiratory tract infection.

Case 2. R. D. was born of healthy parents. A cousin of the father died as a young adult and was said to be a dwarf with normal intelligence. The patient walked at 15 months and developed speech during the second year of life. A gibbus, involving the L-1 vertebral body, was discovered at age 16 months. He gradually developed deformities of head, trunk, upper and lower extremities typical of Hurler's syndrome and had frequent respiratory tract infections. He was referred to the Massachusetts General Hospital at 16 years of age because of recurrent headaches and diminishing vision. Corneas were thickened and moderately cloudy. Visual acuity was approximately 20/100 to 20/200 with glasses and there were 2 to 3 diopters of papilledema. Cerebrospinal fluid pressure was markedly elevated (480 mm of water) and a pneumo-ventriculogram demonstrated symmetrical enlargement of the lateral and third ventricles. Apical systolic cardiac murmurs were prominent and the spleen was palpable. No focal neurologic abnormalities were found. Alder (Reilley) bodies were present in leukocytes. A formal psychologic evaluation indicated an IQ of 100 and a good fund of general information. A ventriculo-atrial shunt was performed to relieve increased intracranial pressure.

During the subsequent 7 years of life, there was no evidence of decline of intellect but a mild hearing loss occurred. During the 6 months prior to death, the patient developed congestive heart failure which was relentlessly progressive despite therapy with digitalis and diuretics.

Gross Necropsy Findings

The significant necropsy findings are summarized below.

Case 1

In the heart there was thickening and wrinkling of the valve cusps and leaflets, involving particularly the mitral and tricuspid valves, and marked thickening of the chordae tendineae without reduction in length. There was a bilateral bronchopneumonia involving all lobes. The liver was large, pale and flabby with focal congestion. Remaining viscera were unremarkable. The body of D-12 vertebra exhibited absence of ossification of its anterior one third to one half, with characteristic "beaking" of the ossified portion (Fig. 1). The brain weighed 880 gm. Some degree of cerebral atrophy was indicated by a minimal narrowing of the convolutions and widening of the sulci. Leptomeninges were generally thicker and tougher than average. The cerebellum was moderately atrophic, especially the vermis. On coronal section the lateral ventricles were enlarged to twice normal size.

Case 2

The heart showed biventricular hypertrophy and thickening of the cusps and leaflets of all four valves. While the mitral valve (aortic leaflet) showed greatest thickening of both cusps and chordae tendineae with consequent moderate stenosis, the aortic valve cusps were thickened and rigid, producing moderate stenosis. The remaining viscera were congested but otherwise negative. There was absence of ossification of the anterior half of L-1 vertebral body, with this portion being represented by a small cartilage nubbin, producing a gibbus similar to that in Case 1. In addition, costochondral junctions were enlarged and globular and on section, exhibited an irregular line of ossification. The brain weighed 1600 gm and no external abnormalities were noted save for thickening of the leptomeninges over the upper brain stem. The lateral ventricles were slightly enlarged while the third and fourth ventricles and the cerebral aqueduct appeared normal. No specific abnormality was found. The shunt tube which passed through the parietal region into the right lateral ventricle was partially occluded at its proximal end by the choroid plexus and ingrowth of fibrous tissue. The spinal cord appeared normal on gross inspection although the spinal dura was very thick.

Results

Histologic Studies

The histologic findings in the two cases are briefly summarized with notation of qualitative or quantitative differences.

Heart. The thickened valve cusps contained spindle and round cells with abundant finely vacuolated cytoplasm (Fig. 2). Similar cells were present in the annuli, in the widened adventitia of the coronary vessels and occasionally in their thickened intima.

Liver. A marked difference was noted in the two cases. In case 1 the parenchymal and Kupffer cells were markedly vacuolated, and in case 2 the section was normal except for chronic passive congestion (Figs. 3 and 4).

Spleen. The sinusoidal lining cells were prominent (in both cases), containing abundant foamy cytoplasm.

Kidney, Adrenal, Salivary Gland and Pancreas. Were unremarkable.

Colon. In both cases the tunica propria, submucosa and muscularis contained foam cells similar to those seen in the heart. In addition the ganglion cells of the myenteric plexus in Case 1 were prominent with vacuolated cytoplasm.

Lymph Node and Bone Marrow. The peripheral sinusoids were filled with the foam cells and in Case 1 they almost completely replaced the

normal lymph node structure. Similar cells were also noted focally in the bone marrow in both cases.

Pituitary. The pars anterior of both cases contained epithelial cells with foamy, greatly expanded cytoplasm (Fig. 5). These cells were more abundant in Case 1.

Thyroid. In Case 1 there was basal vacuolization of the epithelium. Case 2 was unremarkable.

Eye. Only the posterior segment of the eye in Case 2 was available for study. The sclera contained numerous spindle to round cells with abundant vacuolated cytoplasm separated by dense collagen bundles. The retina was unremarkable.

Brain. In both cases the leptomeninges were thickened by an increased amount of collagen, prominent reticulated fibroblasts and foam cells. In addition the adventitia of the parenchymatous blood vessels in the centrum semiovale and in the cerebellar white matter was markedly expanded in the form of a lacy network (Fig. 6). In Case 1 neurons with distended and vacuolated cytoplasm were seen throughout the central nervous system. These abnormalities were especially prominent in the dentate nucleus of the cerebellum, Purkinje cells, pyramidal cells of the cerebral cortex and anterior horn cells of the spinal cord (Fig. 7). Additionally the dendritic processes of the Purkinje cells were swollen forming the so-called "torpedo" processes.[6] In contrast, Case 2 (normal mentality) demonstrated no changes in the neurons. However, the unexpected finding of degeneration of fiber tracts in the spinal cord involving anterior, lateral and posterior funiculi was observed. Where myelinated fibers were most severely depleted there was a mild astrocytic gliosis. Silver stains of the axis cylinders suggested a relative sparing when compared with the loss of myelin sheaths. These changes are presently under further study.

Polysaccharide Studies

The dioxane fixed double embedded tissue exhibited cells containing granules demonstrating metachromasia with alcoholic toluidine blue, PAS negativity and solubility in distilled water. The granules were unaffected by pyridine or chloroform-methanol extraction. When paraffin sections were treated with cetyltrimethylammonium bromide the granules were no longer soluble in aqueous solutions and demonstrated both metachromasia with 1 per cent aqueous toluidine blue and positive staining with alcian blue and dialysed iron reactions. Granules with these characteristics were almost invariably intracellular and found throughout the body as outlined below.

Heart. The vacuolated cells of valve, annuli fibrosi and vascular ad-

ventitia contained the granules (Fig. 8). Rare granules were also seen in myocardial fibers.

Spleen. The sinusoidal cells were sharply outlined by cytoplasmic deposits (Fig. 9) and the fibrocytes of capsule and trabeculae, while unremarkable in H & E preparations, also contained the granules.

Liver. Parenchymal and Kupffer cells in Case 1 were markedly distended by these AMP deposits (Fig. 10). In case 2 the granules were present only in Kupffer cells (Fig. 11). In addition the liver cord cells in Case 1 contained abundant PAS positive deposits which were removed not only by diastase digestion but were also soluble in the saline controls.

Kidney. Rare metachromatic granules were present in the collecting tubules, convoluted tubules and extracellularly in the hyalinized stroma of the pyramids.

Adrenals. The granular AMP deposits were seen not only in the cytoplasm of the cortical cells but in the lining endothelium of the sinusoids and fibrocytes of the capsule (Fig. 12). The accumulations were more abundant in Case 1.

Pancreas. In Case 1 dioxane fixed material was not available for study and in Case 2 no granular deposits were seen.

Salivary Glands. Occasional ducts contained columnar lining cells with the granular cytoplasmic deposits (Fig. 13).

Colon. The cytoplasm of the foam cells noted throughout the bowel wall was distended with granules (Fig. 14).

Lymph Nodes and Bone Marrow. There was abundant cytoplasmic deposition of the metachromatic granules in foam cells.

Pituitary. Granules were present not only in the epithelial cells but in the cytoplasm of sinusoidal cells and the supporting connective tissue. With PAS orange G differentiation, the basophils seemed the most frequent epithelial cell involved.

Thyroid. In Case 2 no accumulations were present. Properly prepared tissue was not available for study in Case 1.

Parathyroid. No granules were present in Case 2. Dioxane fixed tissue was not available in Case 1 but the granules noted in the formalin fixed H & E stained sections demonstrated metachromasia with toluidine blue, alcian blue positivity and PAS negativity.

Testis. In both cases the foam cells and occasional fibrocytes in the interstitium contained granules (Fig. 15) and in Case 1 this was the case with occasional Sertoli cells as well.

Cartilage. The chondrocytes and perichondrial fibrous connective tissue cells were distended with cytoplasmic granules (Fig. 16).

Eye. The prominent vacuolated cells in the sclera contained abundant fine granules (Fig. 17).

Brain. In the leptomeninges the fibrocytes and foamy macrophages contained the granules (Fig. 18). The endothelium of capillaries and expanded adventitia and endothelium of larger parenchymatous vessels were similarly involved (Fig. 19). Deposits in the nerve cells were restricted to the swollen dentritic processes of the Purkinje cells in Case 1 (Fig. 20).

Lipid Studies

The fresh frozen material in Case 1 demonstrated lipid accumulation at various sites. The substance was Sudan black positive, and demonstrated PAS positivity which was reversed by prior acetylation but unaffected by prior diastase digestion. The PFAS reaction was negative and Baker's stain for phospholipids gave a gray black color. Aqueous toluidine blue developed γ-metachromasia and methylene blue binding began at pH 6.0. Alcian blue and Nile blue sulfate staining developed a deep blue color. The Bial test was negative except in the central nervous system where a weak positive reaction was obtained. Prior extraction of the frozen sections in chloroform-methanol or pyridine removed the material but it was unaffected by extraction in distilled water. Deposits of this character were not present in Case 2 or in normal control cases studied.

Heart. Within the valve occasional cells contained this material, particularly the spindle cell forms.

Spleen. Occasional sinusoidal cells contained cytoplasmic inclusions.

Liver. Both the liver cord cells and Kupffer cells contained the abnormal lipid deposits.

Kidney. The convoluted tubules focally demonstrated deposits in epithelial cells (Fig. 21).

Colon. The ganglion cells of the myenteric plexus were distended with the abnormal lipid (Fig. 22).

Testis. Occasional Sertoli cells in the testis contained small cytoplasmic deposits.

Cartilage. In addition to occasional droplets of neutral fat there were cytoplasmic globules of the abnormal lipid (Fig. 23).

Brain. The leptomeningeal foam cells, ballooned neurons, and dendritic processes of the Purkinje cells were filled with the lipid substance. The neuronal deposits differed from those noted elsewhere in the body by demonstrating a faintly positive Bial's reaction.

Remaining organs in Case 1 were negative.

Discusssion

We have presented these cases for several reasons. They offer an excellent example of the additional information provided by techniques

for the histochemical demonstration of extremely water-soluble AMP which predominates in this disease. Also in Case 1, a severely mentally retarded patient, both an AMP and an abnormal lipid accumulated while in Case 2, a mentally normal patient, only the abnormal carbohydrate inclusion was noted. (Table I). This provided an opportunity

TABLE I

ACID MUCOPOLYSACCHARIDE AND GLYCOLIPID ACCUMULATION IN HURLER'S SYNDROME

Site	Acid Mucopolysaccharide		Glycolipid	
	Case 1	Case 2 *	Case 1	Case 2 *
CNS Neurons	o	o	4+	o
CNS Vessels	3+	3+	o	o
Meninges	4+	4+	2+	o
Colonic Ganglion Cells	o	o	4+	o
Heart Valves	3+	3+	2+	o
Spleen	3+	3+	2+	o
Liver	4+	1+	2+	o
Kidney	1+	1+	2+	o
Pancreas	—	o	o	o
Intestine	2+	2+	See ganglion cells	o
Testis	2+	2+	1+	o
Thyroid	—	o	—	o
Parathyroid	2+	o	—	o
Adrenal	3+	1+	o	o
Pituitary	3+	2+	o	o
Lymph Node	4+	2+	o	o
Cartilage	3+	3+	2+	o
Vessels	o–2+	o–2+	o	o
Eye	—	2+	—	o

1 to 4+ weakly to strongly positive
o negative
— tissue not available
* Normal mentality

for considering the biochemical and clinical nature of the disease as it related to the abnormal carbohydrate and the abnormal lipid components. In this regard both the similarities and differences noted in the histochemical analysis of the two cases are provocative and worthy of discussion, particularly in the light of data reported by other workers.

Among the similarities noted were the histologic distribution and histochemical properties of the accumulated AMP. This material fulfilled the histochemical criteria for classification as acid mucopolysaccharides. In addition there were two properties not characteristic of normally occurring AMP: marked water solubility and generalized intracytoplasmic localization. The degree of water solubility even following fixation in various non-aqueous media suggested the absence of protein binding and possibly minimal polymerization.[21]

The origin of the AMP accumulation remains uncertain. The role of

the liver parenchymal cell as the sole or primary site of synthesis is unlikely since in Case 2, the liver contained deposits only in Kupffer cells. Meyer, Grumbach, Linker and Hoffman [11] suggested the fibrocyte as the cell type responsible for synthesis. They also mentioned the possibility that the vascular wall was the site of production since heparin sulfate and chondroitin sulfate B (the two AMP's known to accumulate in Hurler's syndrome) are both in high concentration in the normal vessel wall.[12] In the material available for study in our cases, we were impressed by the frequency with which AMP accumulated in the form of fine cytoplasmic granules present not only in the obvious "gargoyle" or foam cells but also in fibrocytes throughout the body. These fibrocytes frequently appeared unremarkable in H & E stained preparations. In comparison the accumulations noted in vessel walls, particularly in the endothelial cells, were not as consistent nor as prominent. Recent $S^{35}O^4$ autoradiographic studies [22] have cast doubt on the capacity of normal endothelial cells to synthesize AMP. While the site of synthesis of these compounds remains uncertain our evidence would suggest the ubiquitous fibrocyte as a likely site of synthesis; other sources, however, can not be excluded. In addition, the presence of transition forms between the fibrocyte and gargoyle cells, each containing AMP accumulations, supports the concept that many of these characteristic foam cells are derived from fibrocytes.

In Case 1, which was associated with marked mental retardation, lipid storage was noted in various sites including brain, meninges, intestine, heart valve, kidney, liver, spleen, cartilage and testis. The negative PFAS stain and the reversal of PAS reaction by prior acetylation were inconsistent with an unsaturated lipid as the element responsible for the PAS positivity. The parallel loss of PAS and Sudan black positivity in the fresh frozen material after prior extraction in chloroform-methanol or pyridine suggested a close relationship between the carbohydrate and lipid components. The slightly acidic nature of the carbohydrate component was characterized histochemically by alcian blue positivity and methylene blue binding beginning at pH 6.0. The Bial reaction was negative except in the central nervous system where a weakly positive reaction was noted in affected neurons. The fact that methylene blue binding was not demonstrated below pH 6.0 would suggest that gangliosides were not the accumulated material. These aspects of our observations parallel those of Seitelberger who categorized the material histochemically as a glycolipid, having some characteristics shared by gangliosides and cerebrosides but not fulfilling the criteria for classification as either.

Efforts to relate both the carbohydrate and lipid abnormalities, fre-

quently noted in Hurler's syndrome, to a single biochemical defect have been unsuccessful to date.[14] Some workers have suggested that the primary defect is in the AMP metabolism with the lipid accumulation perhaps secondary to the carbohydrate defect.[21] Despite the wide spread neuronal lipid storage in Case 1, only the swollen dendritic processes of the Purkinje cells contained AMP. It seems unlikely then that the central nervous system lipid accumulation is secondary to storage of AMP in neurons. Additionally, with the exception of the Purkinje cell processes in Case 1, the central nervous system AMP accumulation was identical in both cases, being restricted to the fibrous connective tissue elements and the vascular endothelium. The absence of a stainable abnormal lipid in the central nervous system and viscera in Case 2, further suggests the probability that the carbohydrate and lipid abnormalities are separate but frequently associated defects. Recently cases of Hurler's syndrome have been noted in association with metachromatic leukodystrophy,[23] a disease characterized by the accumulation of the sulfate ester of cerebrosides (sulfatide) in both the central nervous system and viscera.[24] Histochemically these sulfatides have characteristic properties including a unique red-brown metachromasia with acidic cresyl violet. Thus it seems that Hurler's syndrome may on occasion be associated with the deposition of at least two different abnormal lipids. The possibility that a variety of different lipids may be deposited in association with Hurler's syndrome, may account for the histochemical and biochemical differences in lipid analyses reported from case to case. It seems also that the carbohydrate defect is the consistent one and is responsible for many of the classic symptoms and signs with the possible exception of mental deficiency.

The occurrence of Hurler's syndrome without mental retardation, while unusual, has been occasionally reported. While the amount of material available for study was limited, our findings would suggest that rectal biopsy might be of particular value not only in distinguishing Hurler's syndrome by the presence of AMP storage cells in the intestinal wall, but also in predicting the likelihood of normal mental development by the presence or absence of lipid storage in the ganglion cells. An important aspect of such a procedure would be the proper handling of the biopsy tissue as outlined here, in order to insure the demonstration of both the lipid and acid mucopolysaccharide constituents, if present.

Summary

Two patients with Hurler's syndrome, one with normal intelligence, served as the basis of histochemical analysis of the abnormal deposits. While in the mentally retarded patient both acid mucopolysaccharides

and an abnormal glycolipid were noted to accumulate in the central nervous system and viscera, the patient with normal intelligence demonstrated only the acid mucopolysaccharide accumulation. It is suggested that the acid mucopolysaccharide and glycolipid deposits may represent separate but frequently associated abnormalities. With the exception of mental deficiency, the acid mucopolysaccharide defect appears to be the one responsible for the classic manifestations of Hurler's syndrome. On a morphologic basis the fibrocyte seems to be the most likely site of AMP synthesis. Rectal biopsy is suggested as both a diagnostic and prognostic aid, verifying the presence of Hurler's syndrome by the acid mucopolysaccharide in foam cells of the colonic wall and predicting the likelihood of normal mental development by the absence of lipid storage in the ganglion cells. The necessity of proper tissue preparation is stressed.

REFERENCES

1. JERVIS, G. A. Gargoylism (lipochondrodystrophy); study of 10 cases, with emphasis on formes frustes of disease. *Arch. Neurol. & Psychiat.*, 1950, **63**, 681–712.
2. WOLFE, H. Techniques for the histochemical localization of extremely water soluble acid mucopolysaccharides. *J. Histochem.* (In press)
3. HUNTER, C. A rare disease in two brothers. *Proc. Roy. Soc. Med.*, 1917, **10**, 104–116.
4. HURLER, G. Über einen Typ multipler Abartungen, vorwiegend am Skelettsystem. *Ztschr. Kinderh.*, 1919, **24**, 220–234.
5. ELLIS, R. W. B.; SHELDON, W., and CAPON, N. B. Gargoylism (chondro-osteodystrophy, corneal opacities, hepatosplenomegaly and mental deficiency). *Quart. J. Med.*, 1936, **5**, 119–139.
6. TUTHILL, C. R. Juvenile amaurotic idiocy; marked adventitial growth associated with skeletal malformations and tuberculomas. *Arch. Neurol. & Psychiat.*, 1934, **32**, 198–209.
7. WASHINGTON, J. A. Lipochondrodystrophy. In: Practice of Pediatrics. BRENNEMANN, J. (ed.). W. F. Prior, Inc. Hagerstown, Md., 1945, **4**, Chapt. 30.
8. LINDSAY, S.; REILLY, W. A.; GOTHAM, T., and SKAHEN, R. Gargoylism. *Am. J. Dis. Child.*, 1948, **76**, 239–306.
9. BRANTE, G. Gargoylism—a mucopolysaccharidosis. *Scandinav. J. Clin. & Lab. Invest.*, 1952, **4**, 43–46.
10. DORFMAN, A., and LORINCZ, A. E. Occurrence of urinary acid mucopolysaccharides in the Hurler syndrome. *Proc. Nat. Acad. Sci.*, 1957, **43**, 443–446.
11. MEYER, K.; GRUMBACH, M. M.; LINKER, A., and HOFFMAN, P. Excretion of sulfated mucopolysaccharide in gargoylism (Hurler's syndrome). *Proc. Soc. Exper. Biol. & Med.*, 1958, **97**, 275–279.
12. MEYER, K.; HOFFMAN, P.; LINKER, A.; GRUMBACH, M. M., and SAMPON, P. Sulfated mucopolysaccharides of urine and organs in gargoylism (Hurler's syndrome). II. Additional studies. *Proc. Soc. Exper. Biol. & Med.*, 1959, **102**, 587–590.

13. HAUST, M. D., and LANDING, B. H. Histochemical studies in Hurler's disease. A new method for localization of acid mucopolysaccharide and analysis of lead acetate "fixation." *J. Histochem.*, 1961, 9, 79–86.

14. LAGUNOFF, D.; ROSS, R., and BENDITT, E. P. Histochemical and electron microscopic study in a case of Hurler's disease. *Am. J. Path.*, 1962, 41, 273–286.

15. MEYER, K., and HOFFMAN, P. Hurler's syndrome. *Arthritis & Rheum.*, 1961, 4, 552–560.

16. UZMAN, L. L. Chemical nature of the storage substance in gargoylism. *Arch. Path.*, 1955, 60, 308–318.

17. DAWSON, I. M. P. The histology and histochemistry of gargoylism. *J. Path. & Bact.*, 1954, 67, 587–604.

18. SEITELBERGER, F. The Position of Gargoylism Amongst the Sphingolipidoses on the Basis of the Histochemistry of Cellular Storage Substances. In: Cerebral Lipidoses. A Symposium. CUMINGS, J. N. (ed.). Charles C Thomas, Springfield, Ill., 1957, pp. 77–91.

19. PEARSE, A. G. E. Histochemistry, Theoretical and Applied. Little, Brown & Co., Boston, 1959, ed. 2, 998 pp.

20. DIEZEL, P. B. Histochemical Study of Primary Lipidoses. In: Cerebral Lipidoses. A Symposium. CUMINGS, J. N. (ed.). Charles C Thomas, Springfield, Ill., 1957, pp. 11–31.

21. BRANTE, G. Chemical Pathology in Gargoylism. In: Cerebral Lipidoses. CUMINGS, J. N. (ed.). Charles C Thomas, Springfield, Ill., 1957, pp. 164–185.

22. STEHBENS, W. E. The production of sulphated mucopolysaccharides by endothelium. *J. Path. & Bact.*, 1962, 83, 337–345.

23. AUSTIN, J. H. Recent Studies in the Metachromatic and Globoid Body Forms of Diffuse Sclerosis. In: Brain Lipids and Lipoproteins and the Leucodystrophies. FOLCH, J., and BAUER, H. (eds.). Elsevier Publishing Co., New York, 1963, pp. 120–133.

24. WOLFE, H. J., and PIETRA, G. G. The visceral lesions of metachromatic leukodystrophy. *Am. J. Path.*, 1964, 44, 921–930.

LEGENDS FOR FIGURES

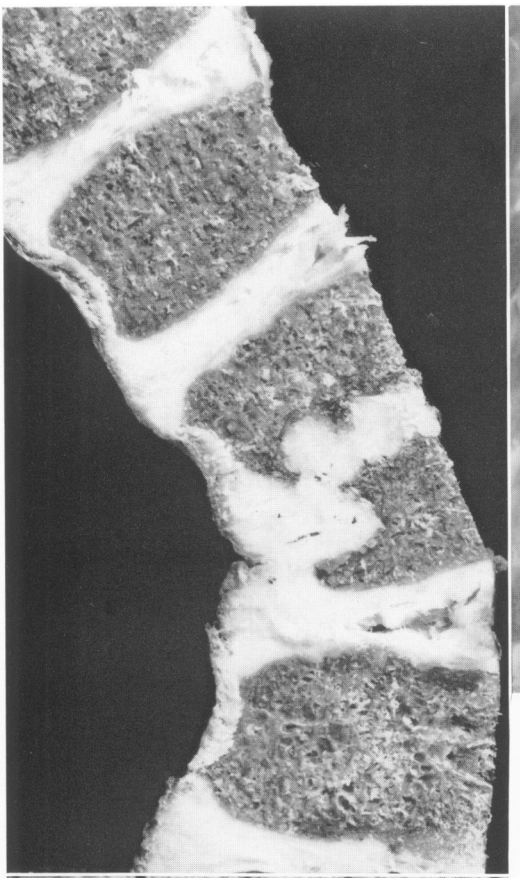
Figures 2 to 7 are prepared from sections fixed in formalin and stained with hematoxylin and eosin; Figures 8 to 20 are prepared from tissue fixed with dioxane and stained with alcoholic toluidine blue.

FIG. 1. A frontal section of gibbus illustrates the absence of ossification in the anterior one-half of D-12 vertebra with beaking of the ossified portion.

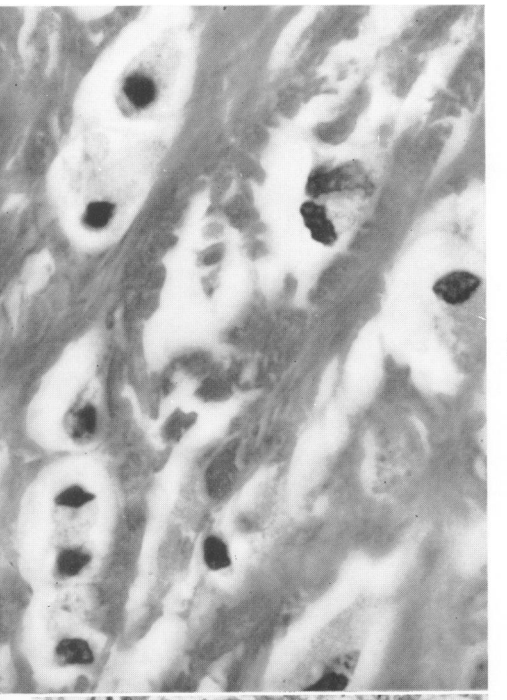
FIG. 2. Mitral valve, Case 1. The characteristic "gargoyle" cells with expanded foamy cytoplasm and deeply staining nuclei are in abundance. × 810.

FIG. 3. Liver, Case 1. There is marked cytoplasmic vacuolization of liver parenchymal and Kupffer cells. × 220.

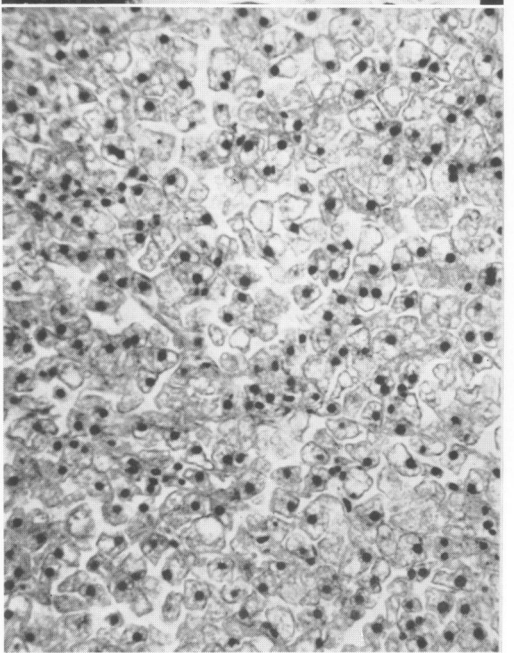
FIG. 4. Liver, Case 2. Histologic structure is essentially normal. × 200.



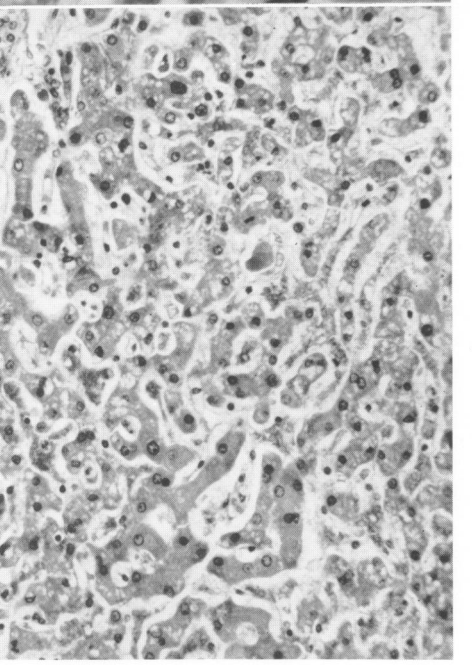
1



2



3



4

FIG. 5. Pituitary, Case 2. Occasional cells exhibit abundant vacuolated cytoplasm intermixed with normal cellular constituents. × 840.

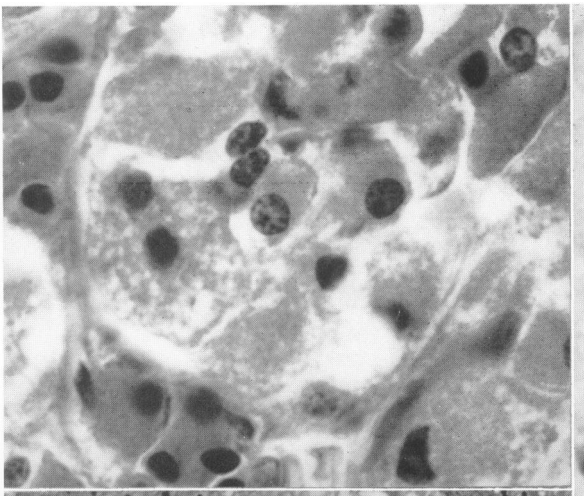
FIG. 6. Parenchymatous blood vessels in the centrum semiovale, Case 1. The adventitia is expanded in the form of a lacy network of fibrous connective tissue. × 130.

FIG. 7. Purkinje cell, cerebellum, Case 1. Cytoplasm is foamy and expanded. × 900.

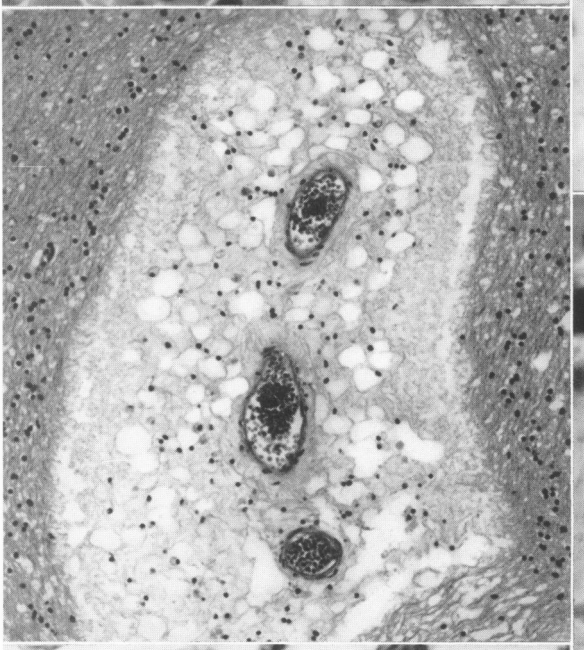
FIG. 8. Mitral valve, Case 1. Cytoplasmic metachromatic water soluble acid mucopolysaccharide is illustrated. × 810.

FIG. 9. Sinusoidal lining cells, spleen, Case 2. Acid mucopolysaccharide granules are shown. × 1,140.

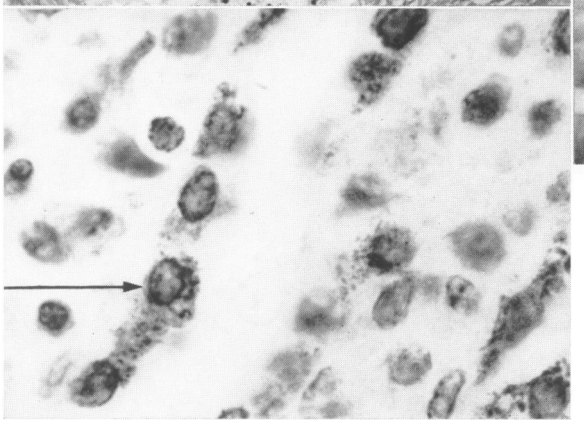
5



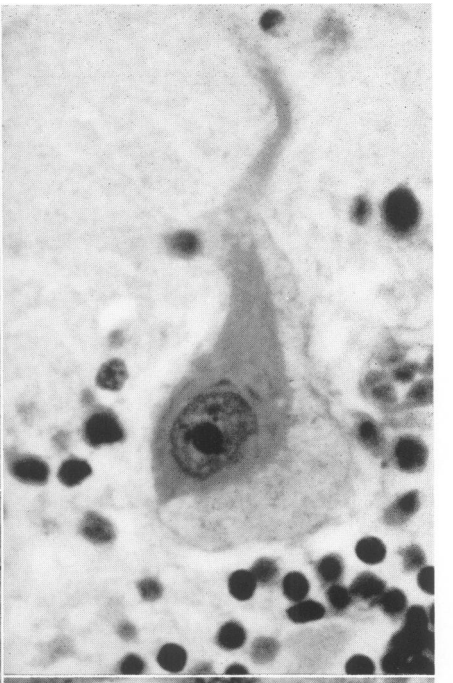
6



9



7



8

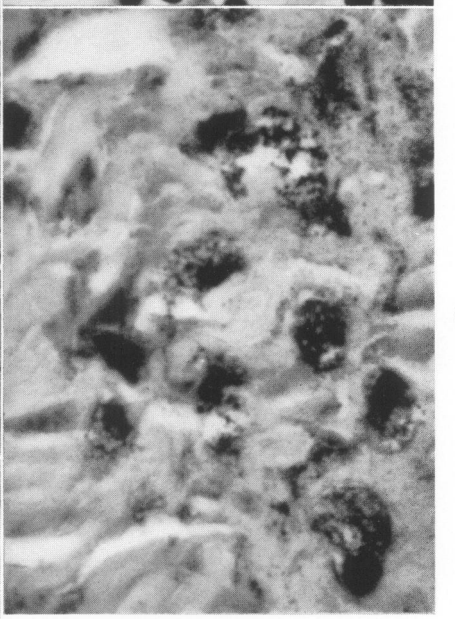


FIG. 10. Liver, Case 1. Marked intracellular accumulation of acid mucopolysaccharide granules is shown. × 300.

FIG. 11. Liver, Case 2. Granules are restricted to the Kupffer cells. × 320.

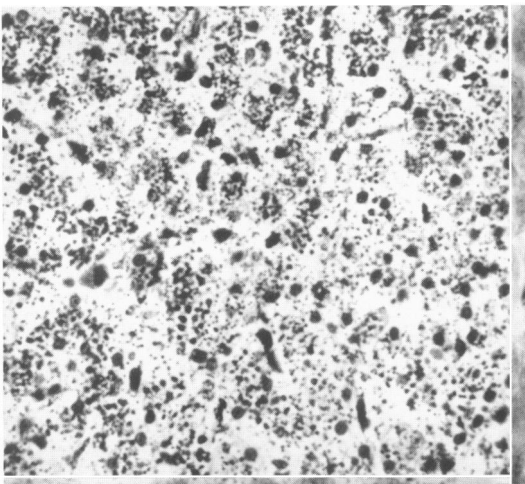
FIG. 12. Adrenal, Case 1. Abundant intracellular deposits of the acid mucopolysaccharides appear in the cortex. × 830.

FIG. 13. Submaxillary gland, Case 1. Columnar epithelium lining the ducts contains cytoplasmic deposits of acid mucopolysaccharides. × 500.

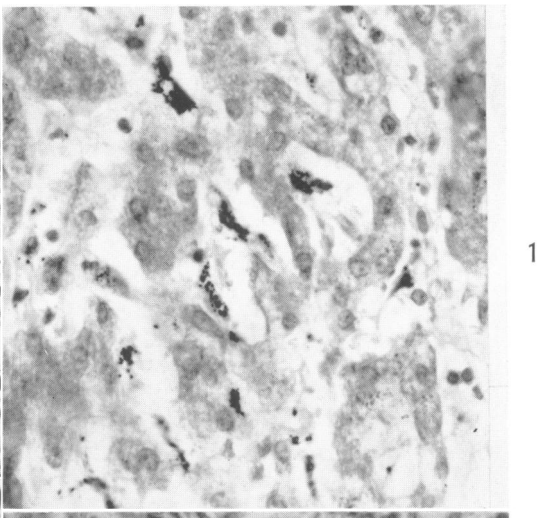
FIG. 14. Colonic mucosa, Case 2. Darkly stained cells infiltrate the tunica propria diffusely. Their cytoplasm contains granular deposits of acid mucopolysaccharide. × 200.

FIG. 15. Testis, Case 2. There is active spermatogenesis. Cells in the interstitium are filled with deeply staining metachromatic granules (arrow). × 470.

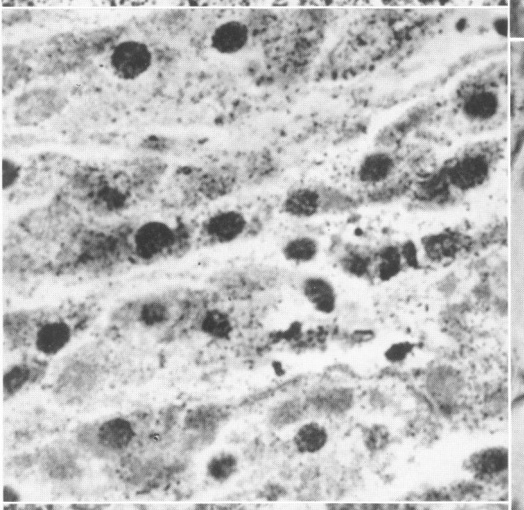
10



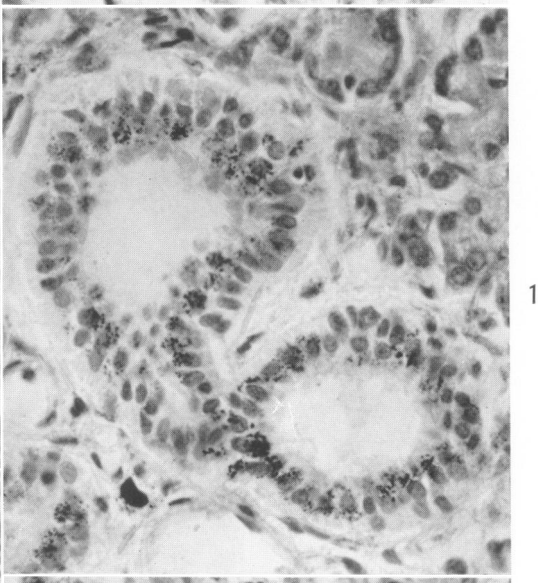
11



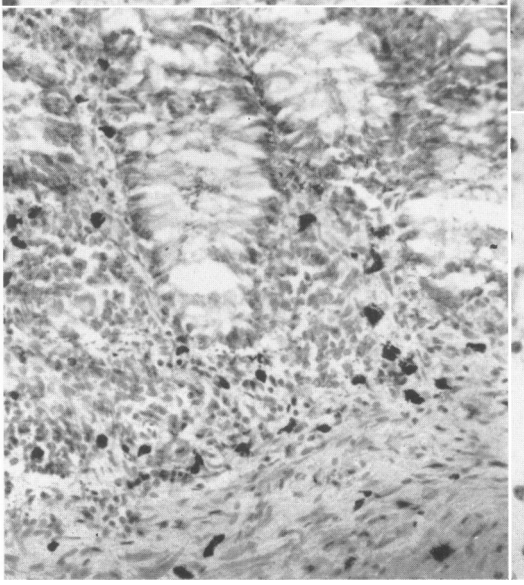
12



13



14



15

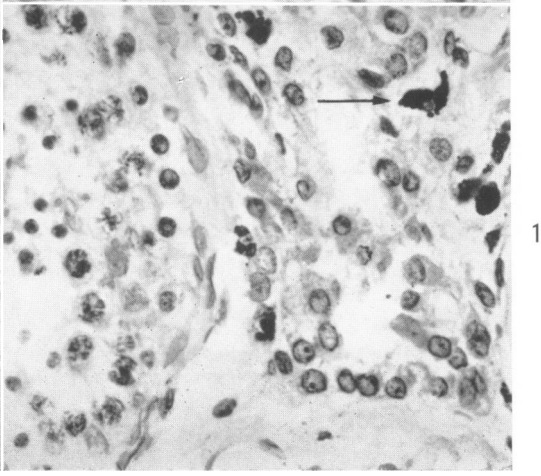


FIG. 16. Articular cartilage from humerus, Case 1. The articular surface is to the right. A great abundance of cytoplasmic acid mucopolysaccharide deposition is manifest. × 930.

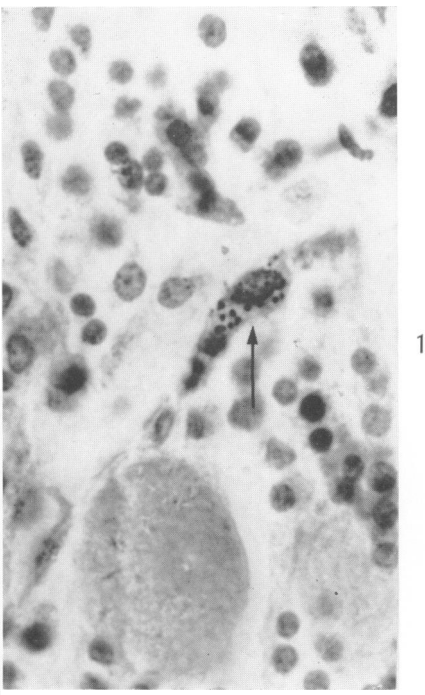
FIG. 17. Sclera, posterior aspect of eye, Case 2. Both spindle cell forms and characteristic "gargoyle" cells contain granular deposits of acid mucopolysaccharide. × 950.

FIG. 18. Cerebellum, Case 1. Thickened leptomeninges are included. At this magnification each dark focus represents a cell filled with granules of acid mucopolysaccharide. × 150.

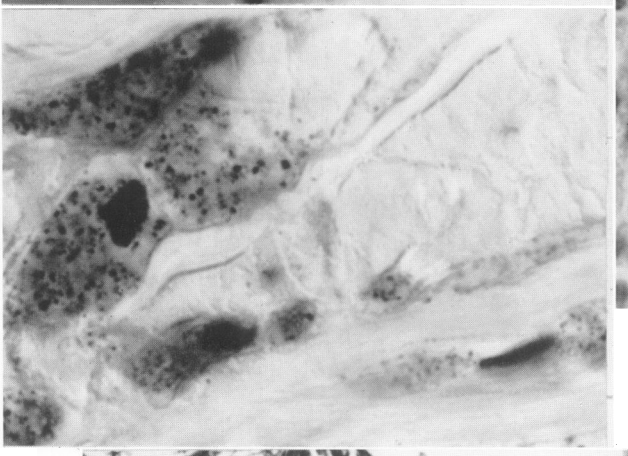
FIG. 19. Cerebellum, Case 2. Fine granular deposits appear within the endothelial cell of a small vessel. × 890.

16

19



17



18

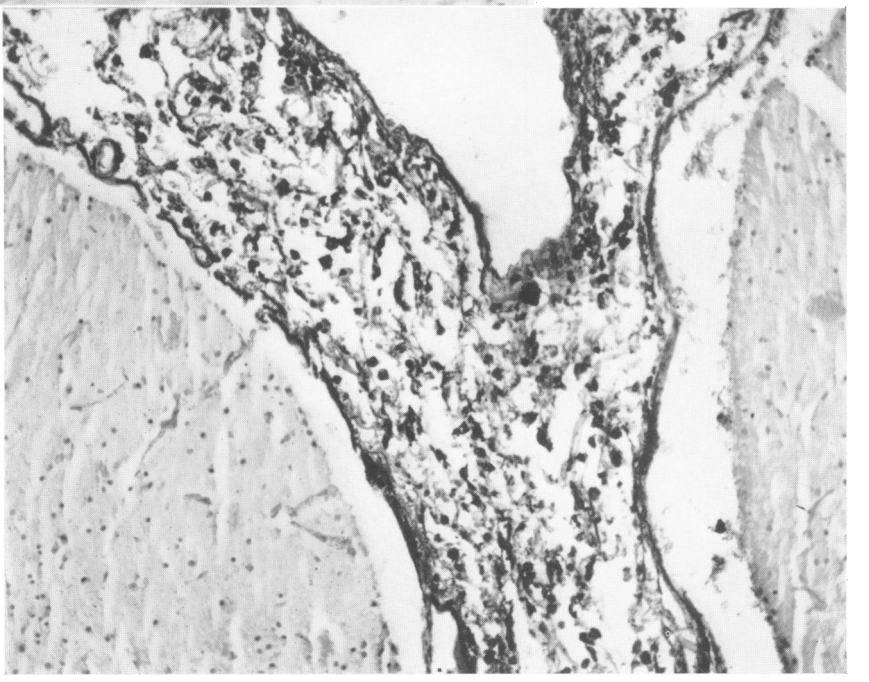


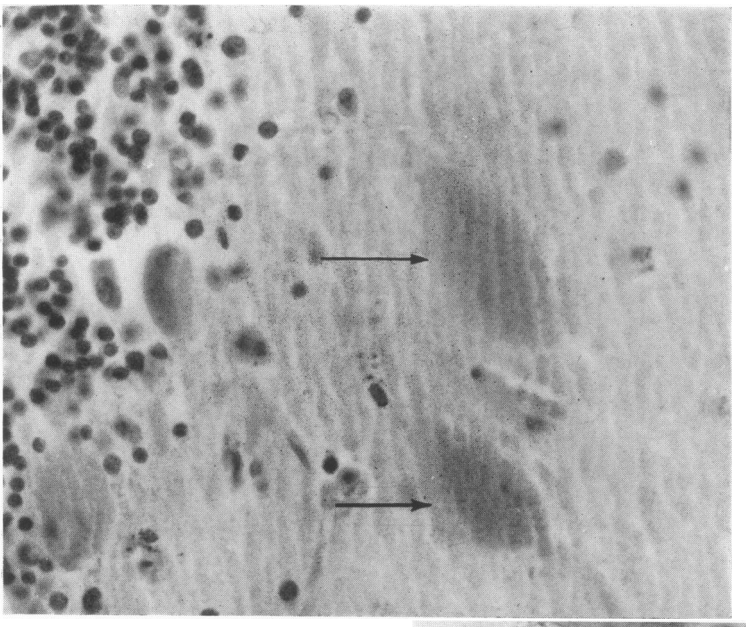
FIG. 20. Cerebellar cortex, Case 1. The two darkly staining areas represent the swollen dendritic processes of Purkinje cells. × 470.

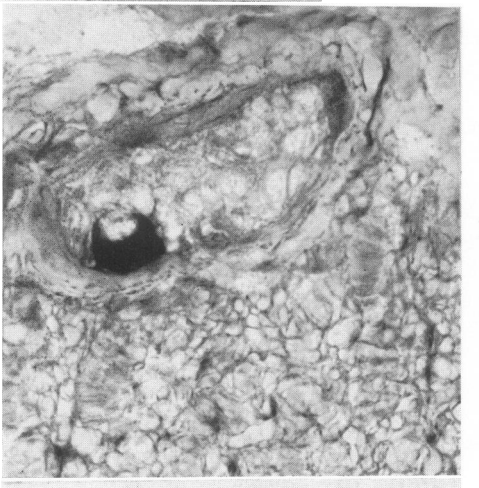
FIG. 21. Kidney, Case 1. Glycolipid is present within the convoluted tubules. Fresh frozen section, methylene blue binding at pH 6.0. × 160.

FIG. 22. Colonic wall, Case 1. The cytoplasm of a ganglion cell in the myenteric plexus is distended. Fresh frozen section, PAS Orange II stain. × 450.

FIG. 23. Costal cartilage, Case 1. Lipid has accumulated within the cytoplasm. Fresh frozen section, Sudan black B stain. × 610.

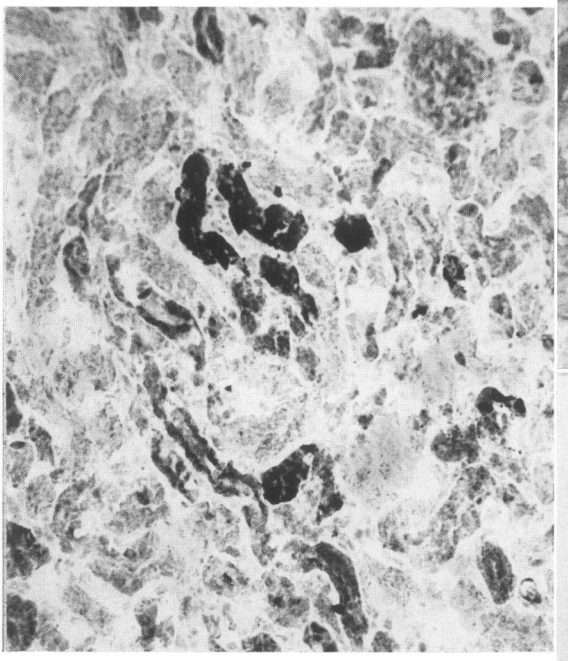
20

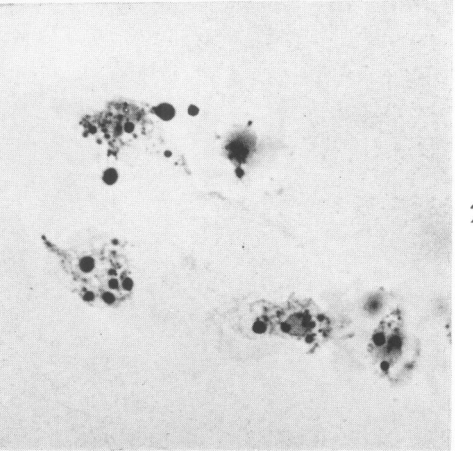




22

21





23