Wolman's Disease

A Rare Lipidosis with Adrenal Calcification

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In 1956 Abramov, Schorr, and Wolman from Israel described a 2-month-old female infant of Persian-Jewish extraction, whose parents were first cousins, who died from a lipidosis in which triglycerides and cholesterol were deposited in various tissues, and in whom the adrenals were extensively involved and diffusely calcified. In 1961 they reported an identical disease in two other sisters of the same family. Crocker *et al.* (1965) reported the first 3 cases of this disorder to be recognized in America. They were not related and were of widely different racial origin. The male patient in their report had a brother who died of an apparently similar disease. In 1966 the first case in Japan was described by Konno *et al.* in a 2-month-old female infant who had 3 sibs, 2 of whom had succumbed after a clinically similar illness.

In a review of the literature, Wolman *et al.* (1961) suggested that single, similarly affected, infants may have been reported as cases of Niemann-Pick disease by Alexander (1946) from New Zealand and by Dienst and Hamperl (1927) from Austria. In a discussion on Gaucher's disease reported in the Scandinavian literature, Henschen (1926) described a 7-week-old infant with 'Niemann's' disease in which the adrenals were extensively involved, but calcification was not mentioned. Caffey (1961) illustrates the adrenal calcification in an infant who died of a disease of the 'Niemann-Pick' type. It seems probable that these are all cases of 'Wolman's' disease.

This paper reports two further examples of this disorder; the first patient, a female infant of Irish descent, was diagnosed during life. The diagnosis was made in the second patient, an English boy, after a review of biopsy and necropsy material previously reported as an unclassified lipidosis.

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Case Reports

Case 1. A female infant was born at term after a normal pregnancy, and weighed 3.18 kg. Her parents are unrelated and of Irish descent; a 7-year-old brother is well. She thrived normally until the age of 5 weeks (weight 3.78 kg.) when she began to pass loose, offensive stools from which specific *Esch. coli* O126 were isolated. Because of continuing diarrhoea, weight loss, and hepatosplenomegaly, she was admitted to The Hospital for Sick Children, at the age of 6 weeks. On examination she was bright and alert, undernourished, and moderately dehydrated. Her abdomen was distended, the liver was palpable 5 cm. and the spleen 1 cm. below the costal margin. There were no other abnormal clinical signs. Investigations showed: Hb 12.4 g./100 ml., leucocyte count 11,600/cu.mm. (P 58%, L 35%, M 7%), erythrocyte sedimentation rate 35 mm./1 hr., fasting blood sugar 79 mg./100 ml., serum total bilirubin 0.2 mg./100 ml., raised serum transaminases (GOT 500, GPT 426 μ units), normal serum electrolyte levels, blood urea, sweat electrolytes, and x-rays of skull and chest. Her urine contained a trace of protein; no sugars were detected on chromatography.

Her symptoms were considered to be due to gastroenteritis; she was treated initially with tetracycline and later with colistin sulphate, after which the specific *Esch. coli* were eradicated. The number of stools decreased but they were still loose and offensive, with pH of 6.0 to 7.0. After an initial weight gain of 0.6 kg. no further increase occurred, and she remained emaciated with a distended abdomen and had a persistent low grade fever, 37.2–37.8 °C. Barium studies of the oesophagus, stomach, and small intestine were normal. Re-examination of the urine revealed an excess of sucrose, but the diarrhoea did not improve on a disaccharide-free diet. Because of progressive enlargement of the liver and spleen the bone-marrow was examined and showed many atypical large histiocytes, with pale vacuolated cytoplasm without intranuclear inclusions (Fig. 1). Haemopoiesis was normal.

By 9 weeks of age Hb had fallen to 5.4 g./100 ml., with normocytic red cell morphology. The anaemia was corrected by a blood transfusion. The leucocyte count varied from 2500 to 7000/cu.mm. (P 40–65%, L 30–48%, M 1.5%, E 1–4%), and on several occasions

FIG. 1.—Case 1. Atypical foamy histiocyte in bone marrow. (Giemsa. $\times 1470$.)

vacuolated lymphocytes were seen in the peripheral blood. There was no thrombocytopenia. At 10 weeks of age a needle biopsy of the liver was performed. The specimen was pale yellow, soft, and very friable. Microscopy showed destruction of the normal pattern, an early pericellular fibrosis, and areas of necrosis. The parenchymal and Kupffer cells were grossly enlarged, vacuolated, and contained sudanophilic lipid and cholesterol. There was no increased acid phosphatase activity of any of the cells. A diagnosis of histiocytosis of unknown type was considered because of the numerous histiocytes in the marrow, and she was treated with prednisolone because of increasing hepatosplenomegaly and general clinical deterioration.

At 10½ weeks of age, x-rays of the abdomen were reviewed and calcification in the region of the adrenals was suspected. Further x-rays clearly showed a finely stippled calcification involving both adrenals which were considerably and symmetrically enlarged (Fig. 2). Skeletal survey only showed poor mineralization. The adrenal calcification together with the clinical features now suggested a diagnosis of Wolman's disease.

Deterioration continued, with increasing hepatosplenomegaly, low-grade fever, and recurrent anaemia. Cyclophosphamide was given in addition to prednisone. Turbidity of the serum in the fasting state was observed, and detailed serum lipid studies are reported together with those of Case 2. In view of the lipaemia, clofibrate was given but no improvement occurred, and she died

at the age of 17 weeks. Necropsy studies are reported together with those of Case 2.

Case 2. The first male child of young, healthy, unrelated English parents, weighed 3.49 kg. at birth. He was well and made normal progress until 4 months of age when he weighed 6.46 kg. He then became reluctant to take solids and passed frequent bulky yellow stools. Mild intermittent vomiting occurred, and abdominal distension developed, with slight enlargement of the liver. During the next 6 months he had a low-grade fever (37.2 – 37.8 °C.), failed to gain weight, remained difficult with feeds, and the diarrhoea persisted despite treatment with a gluten-free diet and prednisone. Abdominal distension increased and the liver enlarged further. Hb fell gradually from 12 g./100 ml. at 4 months to 8.5 g./100 ml. at 10 months, at which age he was transferred to The Hospital for Sick Children.

On admission he weighed 6.35 kg. and had considerable wasting, especially of the limbs and buttocks.

FIG. 2.—Case 1. Lateral abdominal x-ray at 10½ weeks, showing finely stippled calcification of enlarged adrenal.

FIG. 3.—Case 2. Liver biopsy. Photomicrographs. (a) Portal fibrosis and infiltration by lipid-laden histiocytes. The remaining liver cells are swollen and vacuolated. (H. and E. $\times 90$.) (b) Portal tract, showing proliferative ductules and dense infiltration by histiocytes, with vacuolated, foamy or finely crystalline cytoplasm. (H. and E. $\times 300$.) (c) Anisotropic acicular crystals. Frozen section examined under polarized light. ($\times 90$.)

Though there was considerable weakness associated with his very poor nutritional state, he was a bright and alert infant. The fundi were normal and no neurological abnormalities were present. The abdomen was distended, with the liver palpable 5 cm. below the costal margin; the tip of the spleen could just be felt. A small amount of free fluid was present in the abdomen. There were no other abnormal physical signs. Investigations showed Hb 8.5 g./100 ml., reticulocytes 8%, and leucocyte count of 2000–7500/cu.mm. (N 54–80%, L 10–60%, M 3–9%). The bone-marrow was hypocellular, and fairly numerous large histiocytes with pale blue vacuolated cytoplasm and ill-defined cell boundaries were present. Several of these showed haemophagocytosis but in other respects they resembled Niemann-Pick cells. Serum electrolytes, calcium, phosphorus, alkaline phosphatase, and fasting blood sugar were all normal. Total serum bilirubin was less than 0.2 mg./100 ml.; the SGOT was 328 μ units. Total serum protein was 5.4 g./100 ml., and electrophoresis showed reduction in albumin, slight increase in γ -globulin, and moderate increase in β -globulins. Sweat sodium and chloride concentrations were normal. A 3-day fat balance showed 53.3% absorption of fat. No pathogens were isolated from the stools, the pH of which ranged from 8 to 8.5. Urine microscopy was normal, no sugars were present, and the amino acid chromatogram showed a raised excretion of glutamine.

Feeding difficulties persisted, the stools remained bulky, and Hb fell to 7.7 g./100 ml. and was corrected by blood transfusion. At 11 months of age a laparotomy was performed. The enlarged liver was hard and bright yellow. The spleen was twice the normal size but of normal colour. The small intestine was

covered by distended milky lymphatics, and the mesentery was crowded by grossly enlarged lymph nodes of the same colour and consistency as the liver. The kidneys and pancreas felt normal. A wedge of liver and a large (2 $\times$ 1.5 cm.) mesenteric lymph node were removed. Sections of the liver showed that in the central and mid-zones the hepatic cells were enlarged and vacuolated by sudanophilic lipid. The Kupffer cells were unaffected. In the peripheral zones there was complete disorganization and fibrosis; the few remaining parenchymal cells were vacuolated and contained sudanophilic lipid, fatty acid crystals, and cholesterol. The portal tracts were fibrotic and infiltrated by chronic inflammatory cells and lipid-laden histiocytes; the main bile-ducts were normal but there was proliferation of ductules (Fig. 3a, b and c). The lymph node showed almost complete destruction of the normal architecture, only the follicular centres remaining. From the fibrotic capsule, bands of fibrous tissue extended into the node, the remainder of which was replaced by numerous large, vacuolated histiocytes; these contained PAS negative sudanophilic lipid, anisotropic acicular fatty acid crystals, and cholesterol. Some histiocytes were coarsely granular and eosinophilic, showed an increased phosphatase activity, and contained water insoluble, non-refractile, PAS positive sudanophilic material, possibly ceroid (Fig. 4). In some areas the histiocytes had degenerated and liberated their contents. In a few of these areas fine granular calcification had occurred. These appearances excluded a diagnosis of Hurler's, Gaucher's, or Niemann-Pick disease, or a histiocytic reticuloendotheliosis of the Hand-Schüller-Christian type. A diagnosis of an unknown type of lipidosis in which

FIG. 4.—Case 2. Mesenteric lymph node biopsy. Photomicrograph, showing almost complete replacement by vacuolated, foamy or finely crystalline histiocytes. Ceroid present bottom right. (H. and E. $\times 140$.)

there was storage of neutral fat, fatty acids, and cholesterol was made. Results of the serum lipid studies are described separately together with those of the first case.

Clinical deterioration continued with weight loss, diarrhoea, recurrent anaemia, and low-grade fever. Prednisone was given empirically without significant improvement. The liver and spleen enlarged further until the former reached the iliac crest and the latter was below the level of the umbilicus. Death occurred at the age of 14 months.

Necropsy findings are described together with those of the first case.

Serum and Tissue Lipid Studies

Serum. Venous blood, obtained in the fasting state, was analysed from Case 1 at the age of 3 months and from Case 2 at the age of 11 months. Serum total lipid was estimated by the method of De la Huerga, Yesnick, and Popper (1953), total cholesterol by the method of Sackett (1925), phospholipid (lipid phos-

TABLE I
Serum Lipids and Lipoproteins

	Case 1 Female 3 mth.	Case 2 Male 11 mth.	Normal Range
Total lipid (mg./100 ml.)	1200	930	500–640
Total cholesterol (mg./100 ml.)	294	213	150–220
Phospholipid (mg./100 ml.)	327†	294	190–280
Triglyceride (mg./100 ml.)	450	281†	30–123
*Lipoprotein lipid (mg./100 ml.)			
ω	—	65	30–100
β	—	780	250–500
α	—	86	150–280
Ratio β/α ..	—	9.1	1.5–2.5
Pre- β lipid band*	Marked	Marked	Not present

* By paper electrophoresis.

† Calculated from total lipid assuming two-thirds cholesterol to be esterified with fatty acid of average molecular weight 269.

TABLE II
Serum Lipids in Ultracentrifugally Separated Lipoprotein Fractions

Ultra-centrifugal Fraction	Case No.	Total Lipid (mg./100 ml. serum)	Total Cholesterol (mg./100 ml. serum)	Phospholipid (mg./100 ml. serum)
S ₁₅ –100	1	236	32	—
	2	211 (29–114)	40 (5–12)	67 (9–31)
S ₁₀ –15	1	112	28	—
	2	208 (42–79)	47 (13–27)	70 (9–36)
S ₇ –9	1	505	177	—
	2	308 (164–269)	83 (64–97)	97 (45–66)
High density	1	267	81	—
	2	112 (221–302)	19 (57–78)	59 (67–153)

Normal range in brackets.

phorus $\times 25$) by a method adapted from Gomori (1942), and triglyceride by the method of Blankenhorn, Rouser, and Weimer (1961). Paper electrophoresis of lipoproteins was done by the method of Salt and Wolff (1957) and immunoelectrophoresis by the method of Grabar and Williams (1955), using polyvalent anti-human antiserum (Pasteur Institute, Paris). Fractionation of lipoproteins was carried out by density-gradient ultracentrifugation (Cornwell *et al.*, 1961) after removal of the chylomicron layer by preliminary centrifugation of the serum for 30 min. at $10,000 \times g$.

The results are given in Tables I and II. In both children the serum was turbid in the fasting state,

TABLE III
Lipids in Brain, Liver, and Spleen

	Cerebral White Matter†		Cerebral Cortex†		Liver			Spleen		
	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2	Normal ‡	Case 1	Case 2	Normal ‡
Total phospholipid ..	25.49	29.71	27.56	4.3*	6.52	6.89		5.84	7.8	
Total cholesterol ..	8.14	8.99	7.16	0.78*	10.59	11.76	1.5	7.58	3.48	1.5
Esterified cholesterol ..	0.53	0.2	1.28	0.09*	9.31	10.48	0.1	5.62	2.78	0.1
Total hexosamine ..	0.8	0.4	0.8		0.32	0.43		0.41	0.46	
Neuraminic acid ..			0.26							
Water (%) ..	84.9	75.2	85.2		66.3	62.7		69.4	75.9	

Results in g./100 g. dry tissue except * which are by wet weight.

† For normal values see Robinson and Cumings (1967).

‡ Mean values for normal tissue from 3 children.

with an increased total lipid content which was largely accounted for by triglyceride. A marked pre- β -lipoprotein band was demonstrated on paper electrophoresis, and ultracentrifugation confirmed an increase in the very low-density fraction (Sf 15-100) corresponding to pre- β -lipoprotein. The high-density fraction (α -lipoprotein) was normal in Case 1 and reduced in concentration in Case 2. In the latter patient (Case 2), immunochemical studies showed that the α -lipoprotein was identical with that from normal serum and was present in about half the normal concentration (Dr. K. Walton).

Tissues. Portions of brain, divided into white matter and cortex, liver, and spleen from both cases were analysed. Water content, total phospholipid, total and free cholesterol, total hexosamine, and neuraminic acid were estimated (Cumings and Rozdilsky, 1965). Thin-layer chromatography of all tissues from each case was performed by the technique of Müldner, Wherrett, and Cumings (1962). The solvent systems used were chloroform-methanol-water 14: 6: 1 (v/v/v) for phospholipids and chloroform-methanol-10% aqueous ammonia 60: 35: 8 (v/v/v) for gangliosides. In addition, the thin-layer chromatographic method of Brown and Johnston (1962) was used initially for the demonstration of cholesterol and triglycerides, while the method of Freeman and West (1966) was also employed in further confirmation.

Table III shows the results of the estimations while Fig. 5 shows the thin-layer pattern of the triglycerides and cholesterol.

The phospholipids, hexosamine, neuraminic acid, and water content were not significantly abnormal in any tissue. There was a slight excess of cholesterol ester in the cerebral cortex and a definite excess in the liver and spleen in both cases. This is seen in the thin-layer chromatogram which also demonstrates the increased triglyceride in the spleen and liver, though the increase in the spleen was less than that in the liver in the older of the two children (Case 2).

Pathological Features

The morbid anatomical and histological features

were essentially similar in the two cases and will therefore be described together. At necropsy the subcutaneous fat was virtually absent and the muscles thin.

Both *adrenals* were grossly and symmetrically enlarged, hard, and bright yellow, with a normal external pattern. The cut surface showed the cortex to be yellow, with an inner calcified zone, and the medulla was of normal appearance (Fig. 6). On microscopy there was preservation of the zona glomerulosa and of a variable amount of the fasciculata; the cells were swollen, vacuolated, and contained PAS- and luxol fast blue- (Pearse, 1955) negative, sudanophilic lipid, and a trace of cholesterol. The remainder of the cortex was replaced by a broad zone of haphazardly arranged large cells, with palely staining ovoid nuclei, and vacuolated, foamy, or finely crystalline eosinophilic cytoplasm in which numerous acicular anisotropic crystals could be identified. Other cells contained large clefts in the shape of cholesterol crystals. Many of these cells were multinucleate while others had become degenerate, broken down, and had liberated their contents. In the latter regions calcification had occurred; this was mainly finely granular, but in Case 1 there were areas where it was condensed into dense almost crystalline lumps (Fig. 7a and b). In this abnormal zone PAS- and luxol fast blue-negative sudanophilic lipid in fine droplet form and in large globules was present within the cells as well as in the necrotic areas. Large quantities of cholesterol were demonstrated (Weber, Phillips and Bell, 1956). There was extensive fibrosis of the inner two-thirds of the cortex. The medullary cells were inconspicuous but normal.

The *liver* was enlarged and yellow; the cut surface showed loss of the normal pattern and replacement by greasy yellow tissue. The hepatic veins, gall-bladder, and bile-ducts were normal; the portal veins were dilated in Case 2. The microscopical changes were essentially the same as those seen in the biopsy specimens. Portal fibrosis was gross and there was an early cirrhosis. Bile-duct proliferation was present and was marked in Case 2. In addition to the large foamy or vacuolated histiocytes in the periportal zones, the Kupffer cells were swollen, vacuolated, foamy, or had a crystalline appear-

FIG. 5.—Cases 1 and 2. Thin-layer chromatograms of brain, liver, and spleen.

ance. They contained anisotropic and isotropic lipid which stained bright red with Scharlach R, coal black with Sudan Black, and was PAS- and luxol fast blue-negative. Much of this lipid stained green by Weber *et al.*'s (1956) modification of Schultz's method. The presence of cholesterol esters was demonstrated by the digitonin extraction method (Feigin, 1956), and acetyl-phosphatides by the plasma reaction (Hayes, 1949).

The gall-bladder epithelium contained large quantities of sudanophilic lipid, fine deposits of which were present between and in the connective tissue cells and in the adventitia of the blood vessels.

The *spleen* was enlarged, and the cut surface showed replacement of the normal architecture by pale reddish brown tissue which contained numerous fine yellow flecks in Case 1. The majority of the reticulum cells were transformed into large foam cells, and the few preserved follicles were small and compressed. All the foam cells were stuffed by isotropic sudanophilic lipid, cholesterol, and cholesterol esters. In a few cells acicular anisotropic crystals were identified. The *lymph nodes* showed gross enlargement of the mesenteric, coeliac, and para-aortic nodes which were completely replaced by bright yellow tissue; the remaining nodes were normal in size or only slightly enlarged. The thymus was small and firm, weighing

less than 1 g. in both cases. Histologically, all the lymphoid tissue was affected to a varying degree. The mesenteric nodes showed a picture similar to that seen in the biopsy from Case 2. The thymus and the nodes elsewhere were less severely affected, and in the least affected only the sinusoids were filled by lipid-laden foamy vacuolated histiocytes, the follicles being well preserved. The *bone-marrow* was extensively replaced by large foamy or finely crystalline histiocytes.

The entire *small intestine* was thickened and dilated, with a dull, opaque serosa. The mucosal surface resembled bright yellow velvet, and thick yellow villi were easily seen. This appearance was maximal in the duodenum and minimal in the terminal ileum. The small intestinal mesentery was normally fixed but was grossly thickened and pale. The large intestine was apparently normal. The small intestine showed no evidence of mucosal atrophy; the villi were transformed into club-shaped structures by numerous large foamy histiocytes in the lamina propria; similar cells were also present in the lymphoid tissue. All these foam cells were stuffed with sudanophilic lipid and cholesterol. Fine lipid droplets were also present in the overlying epithelial cells, in many connective tissue cells as well as in the vascular adventitia. In Case 2, the supporting cells and neurones of both Auerbach's and Meissner's

FIG. 6.—Case 1. Adrenal cut surface showing very wide cortex which was bright yellow with a calcified inner zone. ($\times 3$.)

plexuses were swollen, with a finely granular cytoplasm, and contained granular sudanophilic lipid.

The *kidneys* showed fine sudanophilic lipid and cholesterol droplets in the mesangial cells of some glomeruli (Fig. 8). The tubules were unaffected.

The *brain* was firm and slightly reduced in size in both cases. The spinal cord appeared normal. There was no evidence of neuronal storage of lipids in the central nervous system, the only abnormality being widespread perivascular cuffing by lipid-laden cells and the presence of finely-scattered lipid droplets in the meninges.

The *skin and connective tissue* of all organs examined contained fine droplets of sudanophilic liquid and cholesterol in and in between connective tissue cells. The *aorta* and large arteries showed some intimal lipid

deposits, their adventitia showed an entirely similar, though more marked, lipid distribution to other connective tissues. The *ovarian* stroma in Case 1 contained small clusters of foamy and vacuolated histiocytes, and in the *testes* of Case 2 there was extensive infiltration of the interstitial tissue with similar cells. Similar lipid-laden histiocytes were present in the *pulmonary* alveoli and septa.

Discussion

There is a remarkable similarity in the clinical features of the cases described by Wolman (Abramov *et al.*, 1956; Wolman *et al.*, 1961), Crocker *et al.* (1965), Konno *et al.* (1966), and the first case in this report. All these infants appeared normal until 2 to 7 weeks of age when they developed diarrhoea and vomiting. The diarrhoea was persistent and uninfluenced by treatment. Abdominal distension, which at times was marked, was associated with increasing enlargement of the liver and spleen. Pallor was consistent with anaemia which at times was severe, and a low-grade fever was present. There was no lymphadenopathy or clinical signs of involvement of the skin, nervous, pulmonary, cardiovascular, or genito-urinary systems. Progressive loss of subcutaneous fat and generalized muscle wasting occurred, and the patients died between 16 and 20 weeks of age. The features of our second case differed only in the time sequence of events, the onset of symptoms being at 3 months and death occurring at 14 months of age.

The familial nature of the disorder is illustrated in the previous reports (Wolman *et al.*, 1961; Crocker *et al.*, 1965; Konno *et al.*, 1966) in which several affected children were present in each family. The available data are consistent with an autosomal recessive mode of inheritance.

The radiological features of the adrenals appear to be unique. All the reported cases have shown punctate calcification of the adrenals which are considerably and symmetrically enlarged and yet retain their normal contour. This is not seen in other conditions where there is adrenal calcification such as neuroblastoma or after adrenal haemorrhage (Neuhauser, Kirkpatrick, and Wientraub, 1965). In classical Niemann-Pick disease calcification of the adrenals is not present. Neuhauser *et al.* (1965) reviewed 26 cases and found no instance of adrenal enlargement or calcification; Crocker *et al.* (1965) and Crocker and Farber (1958) made similar observations, and the experience of this hospital is in agreement. Cases diagnosed as Niemann-Pick disease with adrenal enlargement and calcification are likely, therefore, as suggested by Wolman *et al.* (1961) and Fredrickson (1966), to be examples of Wolman's disease, and liver biopsy will establish

FIG. 7.—Case 1. Photomicrographs of adrenal. (a) Portion of cortex showing, upper left, swollen and vacuolated cells of zona fasciculata, below which are haphazardly arranged large multinucleate cells with foamy or crystalline cytoplasm. In necrotic area, lower right, there is calcification. (H. and E. $\times 145$.) (b) Cells of inner adrenal cortex with vacuolated, foamy or finely crystalline cytoplasm. (H. and E. $\times 360$.)

FIG. 8.—Case 1. Photomicrograph of kidney. Showing vacuolated and foamy appearance of mesangial cells in glomerular tuft. (PAS. $\times 390$.)

the diagnosis. The fact that the presence of adrenal calcification on radiological examination is not essential for diagnosis is emphasized by its absence in our second patient, both during life and at necropsy, though calcification was evident histologically in both adrenal glands.

The morbid anatomical and histochemical findings in our cases and in those described by Wolman, Crocker, and Konno are similar and are different from any other known storage disorders. In our cases the histological picture was characterized by the presence of large histiocytes with vacuolated foamy or finely crystalline cytoplasm scattered throughout numerous organs. Anisotropic acicular crystals could often be identified in those cells which also contained large quantities of sudanophilic, PAS- and luxol fast blue-negative lipid, cholesterol esters and triglycerides, and sometimes acetyl phosphatides. Maximal infiltration by these lipid-laden macrophages was found in the inner half of the adrenal cortex, the mesenteric lymph nodes and the periportal zones of the liver. Many of the cells were multinucleate, while others had broken down and liberated their contents, and it is here that calcification had occurred. This was obvious in the adrenals of Case 1, but was microscopic in the adrenals and mesenteric lymph nodes of Case 2. The association of cholesterol, cholesterol ester, and fatty acid deposition and necrosis obviously determines the site of calcification in this disorder, as it does in a number of other unrelated disease processes such as atheroma and fat necrosis.

Infiltration to a moderate degree was found in the lamina propria of the small intestine, splenic pulp, all lymphoid tissue, thymus, bone-marrow, renal glomerular mesangium, stroma, or interstitial tissues of gonads and septa and alveoli of lungs. The Kupffer cells in the liver were affected at a later stage in the disease. In addition, small lipid-laden histiocytes and fine cholesterol and lipid droplets were diffusely scattered throughout all connective tissues. They were concentrated in the vascular adventitia, the dermis, and submucosa of the intestine and gall-bladder. The central nervous system was unaffected except for fine lipid deposits in the leptomeninges and vessels. The autonomic ganglia were not affected in Case 1. In Case 2 the supporting cells of both Meissner's and Auerbach's plexuses contained masses of lipid which was also present to a lesser degree in the neurones; in view of the extensive intestinal involvement of this case and the longer duration it is possible that these findings were a secondary phenomenon.

The tissue lipid studies in our patients are in agreement with the other reported cases, and again differentiate Wolman's disease from other lipidoses. There is a gross accumulation of esterified cholesterol and of triglyceride in the liver and spleen, but phospholipid and glycolipid levels are normal. In the cerebral cortex of both our cases and in two of Crocker's there was a slight but definite increase of cholesterol ester. In most lipidoses such as Niemann-Pick and Gaucher's disease, structurally abnormal lipids are produced. In Wolman's disease, Rosowsky *et al.* (1965) have shown by gas-liquid chromatography that only genuine cholesterol is present in the non-saponifiable lipid fraction. The mechanism of the enhanced cholesterol storage is not known; presumably it is either due to a defect in cholesterol disposal mechanisms or to a defect in the control of cholesterol biosynthesis. Studies by Slovir, Janic and Naiman (1968) have demonstrated enhanced incorporation of radioactive glycerol and mevalonic acid into the triglyceride and cholesterol ester fractions of red cell membranes, and suggest an increased rate of synthesis.

No detailed studies of the serum lipoproteins have previously been reported. In Wolman's cases the values for total lipid and phospholipid in the first patient, and total lipids and cholesterol in the second, were within the normal range. Of the 3 cases reported by Crocker *et al.* (1965), serum lipids were only examined in 1 patient: at the age of 2 months the total lipid was raised but the levels of cholesterol and phospholipid were slightly reduced and, therefore, by inference the level of triglyceride was raised; thereafter levels of cholesterol and phospholipid fluctuated and gradually declined in the terminal stages. It is difficult to know how much treatment with cholestyramine and thyroxine contributed to the final lipid level. Fredrickson (1966), commenting on the similarity between certain aspects of this syndrome, notably the tissue storage of cholesterol, and Tangier disease (familial α -lipoprotein deficiency), considered that the plasma lipids in the published cases, though somewhat higher than those usually found in Tangier disease, were still compatible with this diagnosis. Our studies rule out this possibility; α -lipoprotein was demonstrated by electrophoretic, immunochemical, and ultracentrifugal techniques, and though it was reduced to about half the normal level in Case 2, this is greatly in excess of the very small amount found in Tangier disease. The striking lipoprotein abnormality in both our patients was an increase in pre- β -lipoprotein (very low-density,

Sf 15-100). This lipoprotein appears in the serum when endogenous triglyceride synthesis by the liver exceeds tissue uptake, and may be present as a secondary phenomenon in a variety of conditions including severe malnutrition. Reduction in α -lipoprotein is a common non-specific finding in ill infants, and is usual when pre- β -lipoprotein is increased (Levy, Lees and Fredrickson, 1966). Though it is tempting to speculate that the serum lipoprotein abnormalities in our patients were causally related to the tissue accumulation of cholesterol, as is probably the case in Tangier disease, we consider that they are more likely to be secondary to the general poor condition and severe wasting of the children.

Attempts at treatment have so far been unsuccessful. Crocker *et al.* (1965) attempted to establish a negative balance for cholesterol by increasing the intestinal excretion of bile acids by the use of cholestyramine, and also gave D-thyroxine to increase cholesterol turnover. They suggest that the poor response they obtained might be explained by the advanced state of the disease, in which hepatic and intestinal dysfunction were already present. Rational therapy must clearly await a better understanding of the basic abnormality.

Summary

Two cases of Wolman's disease occurring in Great Britain are described. This familial lipidosis is characterized by the storage of esterified cholesterol and triglyceride in liver, spleen, reticulo-endothelial system, intestine, and other organs; in particular, the adrenals are extensively and symmetrically involved and often radiologically calcified. Diarrhoea, wasting, and hepatosplenomegaly in early infancy, sometimes accompanied by radiological adrenal calcification, should suggest the diagnosis, which may be confirmed by bone-marrow and liver biopsy.

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REFERENCES

- Abramov, A., Schorr, S., and Wolman, M. (1956). Generalized xanthomatosis with calcified adrenals. *Amer. J. Dis. Child.*, **91**, 282.
- Alexander, W. S. (1946). Niemann-Pick disease: report of a case showing calcification in the adrenal glands. *N.Z. med. J.*, **45**, 43.
- Blankenhorn, D. H., Rouser, G., and Weimer, T. J. (1961). A method for the estimation of blood glycerides employing florasil. *J. Lipid Res.*, **2**, 281.
- Brown, J. L., and Johnston, J. M. (1962). Radioassay of lipid components separated by thin-layer chromatography. *ibid.*, **3**, 480.
- Caffey, J. (1961). *Pediatric X-ray Diagnosis*, 4th ed., p. 736. Year Book Medical Publishers, Chicago.
- Cornwell, D. G., Kruger, F. A., Hamwi, G. J., and Brown, J. B. (1961). Studies on the characterization of human serum lipoproteins separated by ultracentrifugation in a density gradient. *Amer. J. clin. Nutr.*, **9**, 24.
- Crocker, A. C., and Farber, S. (1958). Niemann-Pick disease: a review of eighteen patients. *Medicine (Baltimore)*, **37**, 1.
- , Vawter, G. F., Neuhauser, E. B. D., and Rosowsky, A. (1965). Wolman's disease: three new patients with a recently-described lipidosis. *Pediatrics*, **35**, 627.
- Cumings, J. N., and Rozdilsky, B. (1965). The cerebral lipid composition of the brain in 6 cases of Krabbe's disease. *Neurology (Minneapolis)*, **15**, 177.
- De la Huerga, J., Yesnick, C., and Popper, H. (1953). Estimation of total serum lipids by a turbidimetric method. *Amer. J. clin. Path.*, **23**, 1163.
- Dienst, G., and Hamperl, H. (1927). Über einen Fall von lipoidzelligen Splenohepatomegalie vom Typus Niemann-Pick. *Wein. klin. Wschr.*, **40**, 1432.
- Feigin, I. (1956). A method for the histochemical differentiation of cholesterol and its esters. *J. biophys. biochem. Cytol.*, **2**, 213.
- Fredrickson, D. S. (1966). Wolman's disease. In *The Metabolic Basis of Inherited Disease*, 2nd ed., p. 609. Ed. by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- Freeman, C. P., and West, D. (1966). Complete separation of lipid classes on a single thin-layer plate. *J. Lipid Res.*, **7**, 324.
- Gomori, G. (1942). A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Lab. clin. Med.*, **27**, 955.
- Grabar, P., and Williams, C. A., Jr. (1955). Méthode immuno-électrophorétique d'analyse de mélanges de substances antigéniques. *Biochim. biophys. Acta (Amst.)*, **17**, 67.
- Hayes, E. R. (1949). A rigorous re-definition of the plasmal reaction. *Stain Technol.*, **24**, 19.
- Henschen, F. (1926). In discussion of: Klercker, O.: Contribution à l'étude de la maladie de Gaucher. *Acta med. scand.*, suppl. **16**, 593.
- Konno, T., Fujii, M., Watanuki, T., and Koizumi, K. (1966). Wolman's disease; the first case in Japan. *Tohoku J. exp. Med.*, **90**, 375.
- Levy, R. I., Lees, R. S., and Fredrickson, D. S. (1966). The nature of pre-beta (very low density) lipoproteins. *J. clin. Invest.*, **45**, 63.
- Müldner, H. G., Wherrett, J. R., and Cumings, J. N. (1962). Some applications of thin-layer chromatography in the study of cerebral lipids. *J. Neurochem.*, **9**, 607.
- Neuhauser, B. D., Kirkpatrick, J. A., and Wientraub, B. (1965). Wolman's disease: a new lipidosis. *Ann. Radiol.*, **8** (numéro spécial, Radiologie pédiatrique), 175.
- Pearse, A. G. E. (1955). Copper phthalocyanins as phospholipid stains. *J. Path. Bact.*, **70**, 554.
- Robinson, N., and Cumings, J. N. (1967). Biochemical and histochemical observations on Krabbe's disease. *Acta neuropath. (Berl.)*, **9**, 280.
- Rosowsky, A., Crocker, A. C., Trites, D. H., and Modest, E. J. (1965). Gas-liquid chromatographic analysis of the tissue sterol fraction in Wolman's disease and related lipidoses. *Biochim. biophys. Acta (Amst.)*, **98**, 617.
- Sackett, G. E. (1925). Modification of Bloor's method for the determination of cholesterol in whole blood or blood serum. *J. biol. Chem.*, **64**, 203.
- Salt, H. B., and Wolff, O. H. (1957). The applications of serum lipoprotein electrophoresis in paediatric practice. *Arch. Dis. Child.*, **32**, 404.
- Schiff, L., Schubert, W. K., McAdams, A. J., Spiegel, E. L., and O'Donnell, J. F. (1968). Hepatic cholesterol ester storage disease, a familial disorder. I. Clinical aspects. *Amer. J. Med.*, **44**, 538.
- Sloviter, H. A., Janic, V., and Naiman, J. L. (1968). Lipid synthesis by red blood cell preparations in Wolman's disease (a familial lipidosis). *Clin. chim. Acta*, **20**, 423.
- Weber, A. F., Phillips, M. G., and Bell, J. T., Jr. (1956). An improved method for the Schultz cholesterol test. *J. Histochem. Cytochem.*, **4**, 308.

Wolman, M., Sterk, V. V., Gatt, S., and Frenkel, M. (1961). Primary familial xanthomatosis with involvement and calcification of the adrenals. *Pediatrics*, **28**, 742.

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Addendum

Since this paper was written the parents of our second patient have had two more boys; one is 3 years old and healthy but the second presented at the age of 13 months with a clinical picture similar to that of his

elder brother (Case 2) and identical histological appearances of his liver, intestinal mucosa, and bone-marrow. The same histological appearances have also been seen in the liver of a 5-year-old girl (under the care of Dr. D. G. Cottom) who presented with splenomegaly at the age of 2 years 10 months. It appears, therefore, as if the disorder may have different degrees of expression and is not always fatal in early infancy, as originally described by Wolman. Whether it is related to other cases of hepatic cholesterol ester storage disease (Schiff *et al.*, 1968) is not certain. At present it seems advisable to retain the term Wolman's disease to describe only those children with the features described by Wolman.