Royal Society of Medicine

President Sir John Richardson BT MVO MD

Meeting June 4 1970

Hughlings Jackson Lecture

Inborn Errors of Metabolism in Neurology (Wilson's Disease, Refsum's Disease and Lipidoses)

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Hughlings Jackson (1882) in a lecture given at Worcester said concerning a patient that 'We have to note the tendencies he inherits, as well as to examine him to see how they are particularly evidenced in one branch or twig of a family tree' and it is of interest that this was stated 26 years before Garrod introduced his concept of inborn errors of metabolism (Garrod 1908). Such disorders were understood to mean, at least when I commenced working with Dr J G Greenfield, those diseases which were frequently inherited and in which some special biochemical abnormality was apparent. Thus Garrod had described such conditions as alkaptonuria and cystinuria.

In 1934, a year after I went to Queen Square, Fölling observed that the urine of some mentally defective children in Norway reacted in a specific way with a weak ferric chloride solution, and so he became the first to recognize phenylketonuria biochemically. Since then an increasing number of such disorders have been described and, as each condition was recorded, biochemists attempted to determine the appropriate metabolic pathways involved in normal subjects and where, in patients, these pathways were faulty. Some of these inborn errors and the sites of the metabolic errors will be described, but one more general comment must first be made. Recognizing that frequently a disorder was inherited, geneticists and biochemists discussed means by which inheritance was possible; it has now become a fairly generally accepted principle that coding and the 'master chemical' of the cell are functionally the responsibility of DNA. There have been those who claim that DNA is not of itself responsible but that a DNA polymerase and a synthetase are also involved in the abnormality of the protein molecule and that abnormality is seen by an enzyme deficiency in each particular patient. It may be an enzymic protein or a structural protein or polypeptide arising from the regulatory genes that is in error, but one most not forget that a repressor protein (Bretscher 1968) may be abnormal and that the i gene is the structural gene for this repressor.

Table 1
Inborn errors of metabolism in neurology: some diseases investigated

Hepatolenticular degeneration
Refsum's disease
Lipid disorders
Muscular dystrophy
Pseudohypoparathyroidism
Porphyria
Friedreich's ataxia
Huntington's chorea
Fructose intolerance
Hyperuricæmia
Infantile necrotizing encephalopathy
Abetalipoprotinæmia
Phenylketonuria

My own experience in this field includes diseases listed in Table 1, but this does not include all conditions that could be accepted, nor does it include a number of conditions which are of a doubtful etiology. Three conditions are indicated which will be considered in some detail, together with an indication of the frequency with which they have been seen. The main purpose will be to illustrate the biochemical abnormalities, to indicate schemes of classification as well as to demonstrate the very wide range of opportunity that exists to workers in the field of neurochemistry in relation to human disease.

COPPER AS A TRACE METAL IN DISEASE

A personal interest in hepatolenticular degeneration commenced through a study of various heavy metals in the brain in normal subjects and in various abnormal states. It happened that of my early subjects three patients had died with hepatolenticular degeneration or Wilson's disease and in 1948 evidence was presented of a deposition of copper in liver and brain and in the discussion I said: 'It would seem possible that hepatolenticular degeneration is an inborn error in mineral metabolism' (Cumings 1948). This suggestion was borne out by future observations, even though the exact nature of the disorder is still unknown. Yet through the efforts of research workers, therapy has enabled many patients to survive for considerable periods of active life, demonstrating that treatment may sometimes precede full knowledge.

I shall not repeat the picture of the condition as already presented in 1967 at a Foundation Lecture of the Association of Clinical Pathologists (Cumings 1968) but it is necessary to bring it up to date in a few particulars. I have now made biochemical studies in 95 patients with this condition and Table 2 gives the results. The sex ratio remains the same as previously, 3 males: 2 females. The Kayser-Fleischer ring is the one clinical feature in a patient of more than 12 years of age which is invariably present. The five in whom it was absent were young children with no neurological symptoms or signs apart from features resulting from cirrhosis of the liver. In only three patients have serum copper and cæruloplasmin levels been normal prior to treatment, but each showed the Kayser-Fleischer zone of pigmentation.

Table 2
Hepatolenticular degeneration in 95 cases

	No. of cases
Male	56
Female	36
Sex unknown	3
Kayser-Fleischer ring present	87
Kayser-Fleischer ring absent	5
Serum copper reduced	74
Serum copper normal	8
Serum cæruloplasmin reduced	77
Serum cæruloplasmin normal	5
Serum copper and cæruloplasmin normal	3

Another variation from normal copper metabolism should be mentioned. In primary biliary cirrhosis blood levels of copper and cæruloplasmin are frequently raised and there is an increased urinary copper excretion. Results from ten cases

Table 3
Copper studies in primary biliary cirrhosis

	Serum		*Urine*
Name	*Copper (µg/100 ml)*	*Cæruloplasmin (mg/100 ml)*	*Copper (µg/day)*
Bi	213	39	276
Ga	160	34	280
Fi	117	34	323
Fo	203	44	192
Kr	174	50	162
Ly	183	50	90
Mu	137	42	229
Ma	166	40	268
Sh	253	50	244
St	113	38	194

are recorded in Table 3 but no suggestion as to the mechanism involved can be made.

Normal values have been seen in cystinuria and in infants as well as young children, but in those children with cirrhosis of primary biliary type the findings are similar to those of adults, although not so pronounced.

What is the effect of penicillamine in normal subjects and can one use it as a tool to distinguish normal from abnormal? During the course of my investigations the effect of 900–1,000 mg per day penicillamine on urinary copper excretion in control subjects has been assessed and compared with the results in a selection of patients with hepatolenticular degeneration. The selection has been in choosing those in whom no previous treatment had been given and where it was possible to conduct adequate examinations. Fig 1 shows some of the results obtained. There is a striking difference but I doubt the need for this method of differentiation – a slit lamp examination is easier in adults.

Has therapy really been of value? There is absolutely no doubt that BAL and even more definitely penicillamine, for which we are indebted to Dr J M Walshe, have both prolonged life and rendered the patients more effective citizens. Let me illustrate by describing one patient, originally under the care of Dr D Brinton and more recently of Dr P Gautier-Smith. She was first seen in 1956, aged 30, and is now 45. In 1956 she had had tremor of the hands for 1½ years with defective speech for 1¼ years, and had given up her secretarial duties a year previously. On examination she presented with the typical clinical features of hepatolenticular degeneration including a Kayser-Fleischer ring. Her serum copper and cæruloplasmin levels were considerably reduced and the urine copper was raised. She has been seen and reassessed on fourteen occasions over the past 14 years with little variation in serum levels of copper (*see* Cumings 1968, Fig 4). It should be mentioned that about 10 years ago the liver and spleen became palpable but as recently as last year no œsophageal varices were observable radiologically.

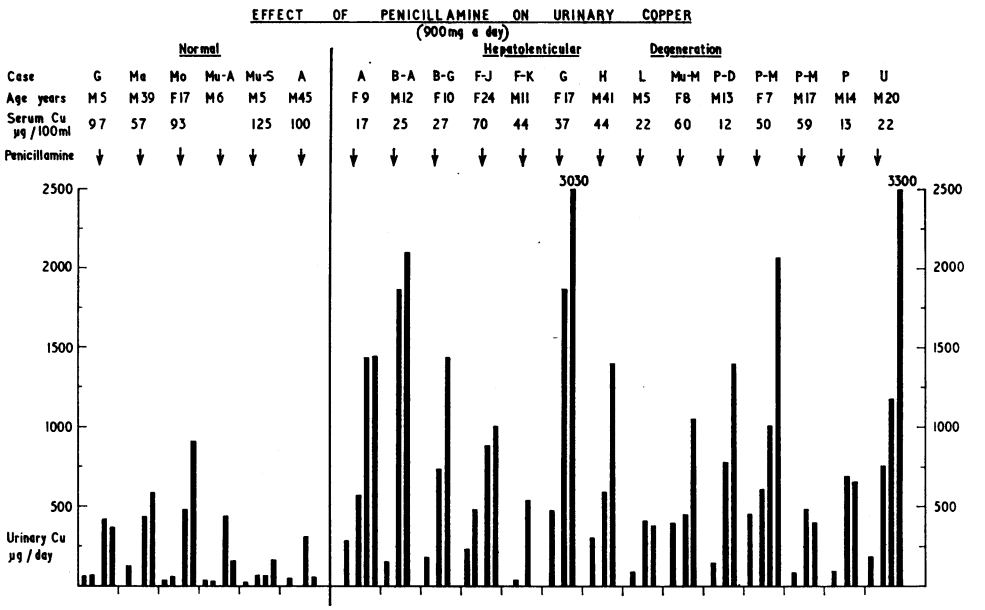

Fig 1 *The effect of penicillamine on excretion of copper in the urine of normal subjects and those with hepatolenticular degeneration*

Her treatment has included BAL, given initially, potassium sulphide and penicillamine. Whereas originally she could not hold a cup without spilling its contents, could not dress, could not do her own cooking or housework, and could not write without holding her wrist with the other hand, all these activities can now be accomplished, and in addition her ability to speak has improved. Even as early as 1960 it was reported that 'this patient has responded fairly well to treatment'. This woman with hepatolenticular degeneration of about 16 years' duration has improved clinically and socially very considerably, even though biochemically there has been no change as evidenced by the copper studies carried out.

However, when one turns to the question of the mechanism of the disorder I doubt if there is anything further to be added to that which has already been written. The fact that it is genetically determined is sound and that copper metabolism is at fault cannot be ignored. It must be assumed that there is an enzymic fault, but which enzyme is involved is still a mystery. Is there an enzyme which participates in the transfer of copper from albumin to the apocæruloplasmin and is this absent or deficient? Is this enzyme also involved in iron transport and can it be related to the increased copper absorption from the gut? Recent work has suggested that there is a closer relationship between iron and copper than has hitherto been suggested (Frieden & Osaki 1969).

REFSUM'S DISEASE

In 1945 and 1946 a condition was described by Refsum which now bears his name. Until about a year ago something under 40 cases had been described and genetic investigations have shown that it is inherited by a rare recessive gene. It is now commonly thought that the enzyme phytanic acid-α-hydroxylase is missing or is deficient.

Klenk & Kahlke (1963) demonstrated that in the blood and some tissues there was an accumulation of 3,7,11,15-tetramethyl-hexadecanoic acid or phytanic acid. This stimulated a number of other workers in an attempt to determine the nature of the defect. Since that time it has been possible to make a personal study of 22 cases: 19 patients with phytanic acid blood levels of from 6·3% to 37·0% of total fatty acids and 3 with CSF levels of 7·0%, 15·6% and 22·5%. Tissues from 6 cases have been examined, 2 without previous blood examinations and in a further case formalin fixed nerve was available. The sex distribution and age range are also shown in Table 4.

Table 4
Refsum's disease

	No. of cases
Total number of cases examined	22
Males (aged 3–44)	12
Females (aged 21–57)	10
More than 1 sibling affected	11 in 5 families
Abnormal blood levels	19
Tissues examined for phytanic acid	6
Lipid studies in tissues	4

All these patients showed the clinical features recorded by Refsum but it must be mentioned that some closely related conditions, with one or other of the striking clinical features absent, have all yielded negative assays.

One typical case under the care of Professor R Gilliatt showed the following features: The patient was almost 24 when she died, having shown an abnormal gait from the age of 15 years as well as being somewhat backward intellectually; when 16 years of age a diagnosis of Friedreich's ataxia and peroneal muscular atrophy was made. Later still there was difficulty in arm movements and slight numbness. Her ataxia became worse and, when examined a few weeks before death, was of a cerebellar type; she showed tapetoretinal degeneration, hypertrophic polyneuropathy with thickened ulnar, sural and great auricular nerves. There was some bulbar weakness, necessitating the use of a respirator for one week before death. The cerebrospinal fluid contained 1·4 g of protein per 100 ml and the serum phytanic acid was 31·3% of total fatty acids.

Table 5
Phytanic acid in nerve as percentage of total fatty acids

Case	*Peripheral*	*Optic*
OH	3·8	–
MW	4·9	–
CC	5·0	–
LS	3·7	5·4
CL	5·7	1·2

The determination of phytanic acid in a nerve at autopsy or biopsy has been made in 5 cases with results seen in Table 5. The results in tissues obtained in 4 cases revealed a large amount of phytanic acid in the liver and heart but much smaller amounts in the brain (*see* Table 6). The results of the analysis of the brain and organs of the patient whose clinical findings were mentioned earlier are those shown in the last column. There are a few interesting features worthy of note. Levels of phytanic acid in the brain are low, but there is as much as 14·9% of phytanic acid (of total fatty acids) in the choroid and pigment layer,

Table 6
Phytanic acid in tissues as percentage of total fatty acids

Patient	OH	CR	LS	CL
Sex	F	M	F	F
Age	57	44	21	23
Cerebral white	1·0	0·1	2·9	1·5
Cerebral cortex	Trace	0·1	3·5	0·5
Eye:				
Choroid and pigment			35·4	14·9
Rods, cones, ganglion cells				4·4
Liver	12·3	34·4	43·5	29·0
Spleen	5·6	2·0	–	–
Heart	25·2	–	–	22·2
Blood	4·4	–	37·0	31·3

Table 7
Phytanic acid and cholesterol in organs in Refsum's disease

	Liver		*Heart*		*Kidney*
Patient	CL	LS	CL	LS	CL
Phytanic acid %:					
In cholesterol ester	64·8	79·5	75·0		88·0
In triglycerides	41·8		58·6		57·5
In lecithin	11·7	10·0	16·8		44·5
Total cholesterol in mg/100 g wet	328 (204)	530	102 (120)	198	424 (175)
Esterified cholesterol in mg/100 g wet	139 (50)	250	31 (10)	25	233 (14)

(normal in brackets)

but only 4·4% in the rod and cone layer of the retina. When the amount of cholesterol, both total and esterified, are estimated in the heart, kidney and liver, there is a higher content of esterified cholesterol in the organs as compared to normal (*see* Table 7). Table 7 also indicates the large amounts of phytanic acid present in cholesterol esters of the organs. It can never be assumed that the accumulation of a product, whether it be phytanic acid in Refsum's disease or phenylalanine as in phenylketonuria, means that the clinical signs are a direct result of such a product, but it does indicate evidence of a metabolic block, as here where there is a suggestion of an inborn error in the degradation pathway for branched chain fatty acids. Initially a defect of omega oxidation of fatty acids was postulated (Eldjarn 1965) but later the lack of a pathway involving a CO_2-fixation mechanism was suggested from the work of Eldjarn *et al.* (1966). More recently the absence, complete or partial, of the enzyme phytanic acid-α-hydroxylase has been suggested by Steinberg *et al.* (1967) as the primary factor involved, as can be seen in Fig 2.

It is of considerable interest that while the organs, the peripheral nerves, cerebrospinal fluid and even parts of the retina contain an excess of phytanic acid, the brain contains only small amounts and the so-called blood brain barrier must be relatively impermeable to phytanic acid. As also shown previously (Skrbic & Cumings 1969), changes are present in liver, heart and kidney, not only as regards phytanic acid content but also in levels of cholesterol. Death of a patient may well be associated with these cholesterol infiltrations rather than an increase in an unusual fatty acid.

Perhaps it would be as well to mention here an important technique in diagnosis even though neurologists may have little opportunity themselves to practise it. Many hereditary disorders can be recognized from very early life; I have examined one still-born fœtus and diagnosed

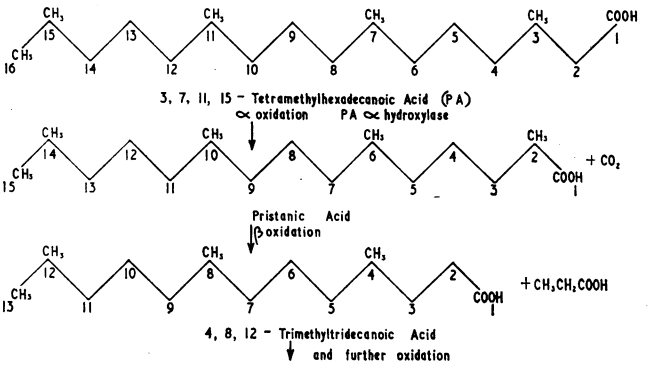

Fig 2 *Phytanic acid degradation shown diagrammatically*

Niemann-Pick's disease. Recently amniotic fluid has been withdrawn by abdominal paracentesis in pregnant women. This is possible from about 10–12 weeks up to the 20th week and 2–8 ml of fluid can be withdrawn. The determination of the sex of the fœtus is simple from the cells which can also be cultured, and from biochemical as well as histo-enzymic studies made on both cells and fluid. It should, perhaps, be pointed out that in only some 66% is there a successful culture and the method is not without its dangers. It is now possible to demonstrate the presence of a formidable array of enzymes including phytanic acid-α-hydroxylase (Uhlendorf & Herndon: *see* Nadler 1969), the absence of galactose-1-phosphate uridyl transferase in galactosæmia (Nadler 1968) and of hypoxanthine guanine phosphoribosyl transferase in the Lesch-Nyhan syndrome (Uhlendorf & Fugimoto: *see* Nadler 1969). In the case of Refsum's disease it seems less likely that this technique has much to offer, but in some of the lipidoses, as will be mentioned later, as well as in Duchenne muscular dystrophy, this procedure could be of the utmost importance and it is a field which has already received considerable attention, particularly in children's hospitals in this country and in Germany.

LIPIDOSES

Another group of metabolic disorders where an inborn error is present is that involving tissue deposition of some lipid. This is a vast subject and investigations of these conditions have been in progress in my laboratory since 1952, and during this period 784 brains have been studied in a variety of clinical conditions. Using a nomenclature in common use, I have listed some of these in Table 8 and for the opportunity to study this material I am indebted to clinicians and pathologists in this country and from many places abroad. Of the twelve abnormal groups there are at least seven conditions now known to be associated with an enzyme defect present from or before birth. It is probable that only the last four will be of different etiology but even this is uncertain.

The initial descriptions and definitions of these conditions were the work of neurologists and physicians, who recorded in detail the signs and symptoms of each patient seen, sometimes, as in Tay-Sachs disease, with assistance from an ophthalmologist. Histologists then proceeded to the anatomical minutiæ of the brain (and retina) in these conditions and the German pathologists as well as Dr Greenfield were particularly prominent. In recent years the electron microscope has enabled workers like Terry and Zeman to demonstrate certain special characteristics. Meanwhile, neurochemists such as Klenk were elucidating the nature of the deposited and stored lipid, followed later by a study of the metabolic pathways of the various lipids. It was a short step to the identification of the enzymes responsible for each metabolic step and from there to theories as to the defect in disease processes. The final step, thus far, is the discovery, as suspected by some already, that the lipid changes in many of these diseases are not localized to the brain but can be detected in many organs or even in biological fluids. It must, I think, be assumed that with

Table 8
Abnormal conditions of lipid nature

	No. of cases
Gaucher	11
Niemann-Pick	13
Fabry	4
Tay-Sachs	17
'Amaurotic familial idiocy' (all forms)	76
Metachromatic leukodystrophy	47
Globoid (cell) leukodystrophy	18
Pfaundler Hurler	18
Multiple sclerosis	47
Sudanophilic diffuse sclerosis	33
Alpers' disease	11
Wolman's disease	4

modern techniques and with the basic chemical knowledge now available it is possible to classify and define these various lipid disorders in an exact manner. However, the understanding of all of these disorders and their final classification will only be arrived at by the joint efforts of all from whatever discipline they come.

Details of the metabolic pathways involved in the metabolism of the various sphingolipids can be found illustrated in various books and journals, but in order to demonstrate them easily prior to recording the results that have been obtained Fig 3 indicates the sites of five enzymes in the pathways concerned and when one enzyme is absent or reduced a disease process can be recognized by specific abnormal biochemical alterations. The stored compounds are glucocerebroside, sphingomyelin, ceramide trihexoside and sulphatide in the first four with a raised cerebroside/sulphatide ratio in the fifth.

The metabolic pathways relating to the gangliosides are rather more difficult and perhaps a few comments on these substances are first necessary. Gangliosides are mainly present in the cerebral cortex, although traces are present in the white matter. It has been shown that they are increased in infancy at the same period of time that myelin is being laid down (Cumings *et al.* 1958). More recently it was demonstrated that they were present in microsomes (Thompson *et al.* 1967) but not normally found in white matter myelin, while in later experiments they were found in synaptosomes. These findings have recently been confirmed by Halaris & Jatzkewitz (1969) in some electron histochemical studies. When the gangliosides were studied by us on thin-layer chromatography it was shown that there were a number of different components (Wherrett & Cumings 1963), each of which has more recently been shown to contain varying molar amounts of hexose, N-acetyl-neuraminic acid (NANA), sphingosine and hexosamine. These various subdivisions of ganglioside have been termed by Svennerholm (1963): G_{M1}, G_{D1a}, G_{D1b} and G_{T1}, containing one or more N-acetyl-neuraminic acid residues. In the metabolic pathway other ganglioside fractions, as G_{M2}, are formed but these are not found in the normal subject except in trace amounts, although in some diseases they are present in larger amounts.

Fig 4 illustrates a metabolic pathway by which gangliosides can be metabolized, commencing with ceramide monohexoside and by means of various enzymes progressing through the monosialogangliosides to the disialoganglioside G_{D1a}, while in G_{D1b}, not shown here, the two NANA components are linked together on one galactose.

Mention was made just now that in some of these disorders there is recognized to be evidence of a widespread abnormality with a lipid deposited in an organ such as the kidney, as well as in the brain, while in some instances the enzymic abnormality present in the brain can also be detected in biological fluids and in tissue culture of skin fibroblasts or in the amniotic fluid of the mother. Thus urine can be examined for sulphatides or for aryl sulphatase in metachromatic leukodystrophy, the blood for hexosaminidase A in Tay-Sachs disease, or an absence of β-galactosidase in some forms of generalized gangliosidosis. These are but a few examples; the real problem is that most have been demonstrated in known and already diagnosed diseases, and each examination requires considerable space, time and expense, so that to scan an unknown condition by all of the methods at present avail-

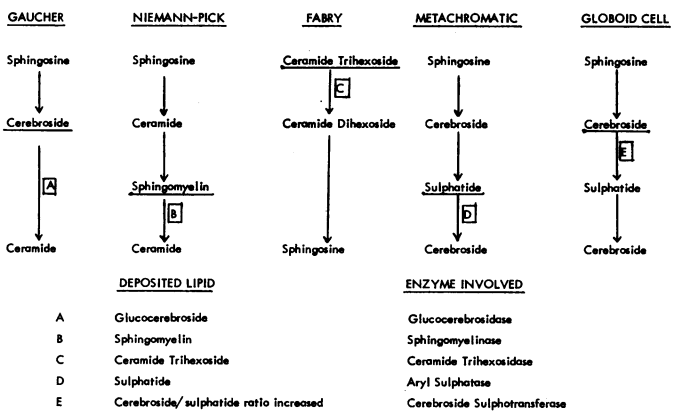

Fig 3 *Diagrammatic representation of metabolic pathways in some lipid diseases with the sites of action of the enzymes involved shown*

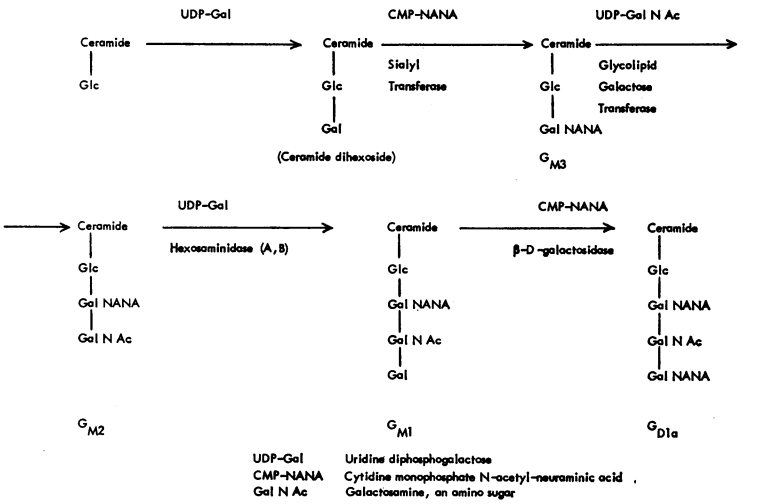

Fig 4 *Metabolic pathway for gangliosides*

able would appear to be possible only in a few laboratories.

We have been engaged in lipid studies since 1952 and in these 18 years a large selection of material has been studied. Table 9 lists some of the major conditions in which cerebral lipids have been assayed, but many diseases are omitted, and only a few can be mentioned in any detail. Table 9 condenses the findings in seven disorders and includes the number of cases personally examined, the lipid abnormality detected in each, the enzyme involved, the site where they can be detected and the degree of reduction of the enzyme in each disease. Previous communications have included discussions on various aspects, both in general clinical and biochemical terms (Cumings 1965) and in basic biochemical aspects (Booth *et al.* 1966, Cumings *et al.* 1968) in a number of these disorders. Some additional comments will be made in one relatively rare condition known as Fabry's disease, for four such cases have been studied and have not yet been published.

Case History

I am indebted to the late Dr P Sandifer for the history of the patient, aged 31 when he died; he, the younger by three years of two brothers, showed when examined nine years earlier, the typical clinical picture of Fabry's disease. There were severe pains of the periphery of the legs and genitalia since childhood, skin angiomata were present and albuminuria was a feature. There was papilloedema in childhood, ulcerative colitis in the teens and a later cerebral vascular accident with a resultant partial aphasia. The older of the two brothers died some four years before the younger, and I am grateful to Professor William

Table 9
Abnormalities in lipid diseases

Disease	*No. of cases*	*Abnormalities found*	*Reported enzyme deficit*	*Site of enzyme*	*Relative % of enzyme*
Gaucher	11	Cerebroside+ (glucose)	Glucocerebrosidase	Skin fibroblasts	3
				Leukocytes	
				Spleen	1
Niemann-pick	12	Sphingomyelin+ G_{M3}, G_{M4} present	Sphingomyelinase	Leukocytes	8
				Liver	5
Tay-Sachs	17	G_{M2}+	Hexosaminidase A	Muscle	0
				Blood	0
Generalized gangliosidosis	9	G_{M1} or G_{D1a}	β-D-galactosidase ?	Brain	30
Metachromatic leukodystrophy	47	Sulphatide+ Cerebroside −	Aryl sulphatase	Leukocytes	10
				Brain	1
Globoid (cell) leukodystrophy	18	$\frac{\text{Cerebroside}}{\text{Sulphatide}}$ ratio+	Cerebroside sulphotransferase		
Fabry	4	Ceramide trihexoside+	Ceramide trihexosidase	Small intestine	0·5

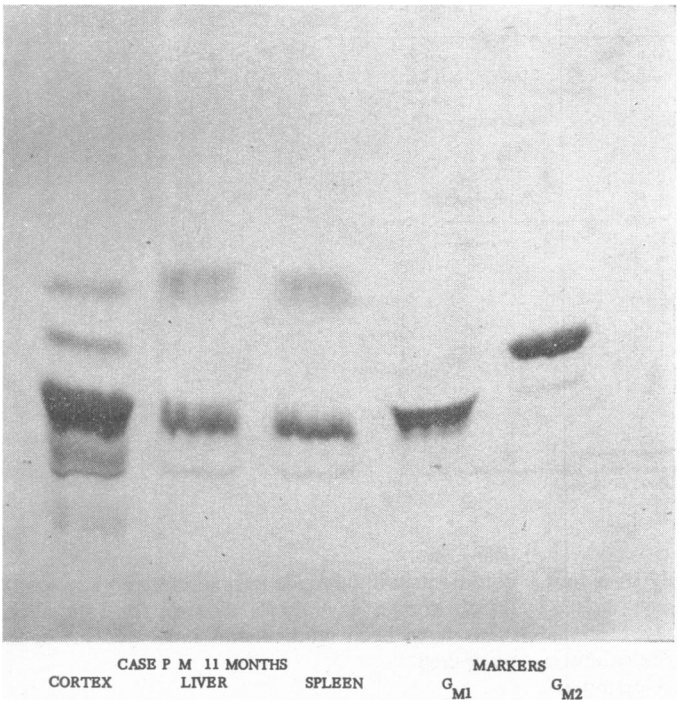

Fig 5 *Thin-layer chromatogram from lipid extract of cerebral cortex, liver and spleen from a case of G_{M1} gangliosidosis*

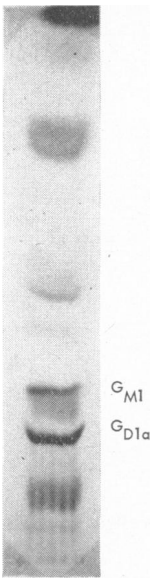

Fig 6 *Thin-layer chromatogram of lipid extract of cerebral cortex in a case of 'amaurotic familial idiocy' to show increase of G_{D1a} and loss of G_{M1}*

McMenemey for the post-mortem specimens from both brothers.

The analysis of the kidneys of both cases, of the liver and the sciatic nerve of one compared with a normal kidney, gave the following results: the thin-layer chromatogram of the lipid extract of the kidney was stained for the ceramide components and analysis of each band for sphingosine content was made by a method previously described (Cumings *et al.* 1968) and comparison made with a normal kidney. It is seen in Table 10 that there is a striking increase in ceramide trihexoside with a reduction in the tetrahexoside component. It should also be mentioned that in the brain no abnormality was detected. The urines of patients with this condition show a similar increase in ceramide trihexoside, present also in at least one parent of two other families.

Table 10

Ceramide hexosides in Fabry's disease: results as percentage of total sphingosine

	Ceramide tetrahexoside	*Ceramide trihexoside*	*Ceramide dihexoside*	*Cerebroside*
GW				
Male aged 31:				
Kidney	2·26	65·1	25·2	Trace +
Liver	Trace	60·7	14·3	3·6
Sciatic nerve	Trace	42·9	2·5	36·5
HW				
Male aged 30:				
Kidney	4·4	69·1	26·3	Trace
Normal control				
Kidney	43·7	44·9	11·3	Trace

The group of disorders commonly known as amaurotic familial idiocy (AFI) will be commented upon as many problems arise as to classification. In 1896 Sachs suggested the term 'amaurotic family idiocy' and defined it as a genetically controlled disorder with certain characteristic clinical features, such as the retinal changes. During the next few years this label covered a variety of conditions, but in 1905 Vogt separated two forms, infantile Tay-Sachs and a juvenile form, an opinion supported by Spielmeyer (1905). Since then clinicians and pathologists have divided up the juvenile form into a number of subgroups including a late or adult form (Kufs 1925). Zeman (1970) has recently presented a historical survey and brought the subject up to date.

Tay-Sachs disease with a raised content of ganglioside in the cerebral cortex was for a number of years considered a single disease entity with a characteristic ganglioside abnormality (Müldner *et al.* 1962). Recently it was demonstrated that in the typical case hexosaminidase A was missing and this is now called Type 1. There appear to be two other types: in Type 2 there is an absence of

Table 11
Ganglioside abnormalities in AFI

Case	Sex	Age	*NANA in cortex (mg/100 g dry tissue)*	Specimen	*Visceral organs affected*
G_{M1} *gangliosidosis*					
Typical:					
DH	F	32	16	Autopsy	+
NP	M	5	50	Biopsy	Cyst in humerus
JP	M	1	70	Biopsy Autopsy	+
PM	F	11 months	41	Autopsy	+
Atypical (G_{M3}, G_{M4} increased):					
KP	M	4½	31	Autopsy	+
PP	M	6	25	Autopsy	+
G_{D1a} *gangliosidosis*					
CG	M	15	32	Biopsy	
LF	F	2	18	Biopsy	
AM	M	5 months	25	Autopsy	–
G_{M2} *gangliosidosis (late onset Type 3)*					
JN	M	5	60	Biopsy	

hexosaminidase A and B (O'Brien 1969) while in Type 3 there is only a partial diminution of N-acetyl-β-D-galactosaminidase and the condition is of a late onset, and tissue from one brain of such a case has been personally examined.

There is evidence that in only the exceptional case of AFI are gangliosides increased or abnormal. Klenk (1939) did not obtain an increased ganglioside content in the cerebral cortex in AFI and, although in general we agree with this view, yet the degree of reduction in phospholipid and sphingolipid content is not matched by an equal reduction in ganglioside in all the cases examined. There have been some exceptions, for in 1964 Landing *et al.* described familial neurovisceral lipidosis, now often known as G_{M1} gangliosidosis. Zeman, from his studies, regards the Batten form as a distinct disorder which he considers to be a neuronal ceroid-lipofuscinosis (Zeman & Donahue 1963, Zeman & Dyken 1969).

It is, therefore, of some interest to put on record that of the 76 cases of so-called amaurotic familial idiocy, 37 have presented since 1962 when we published the first report on the presence of the abnormal Tay-Sachs ganglioside by the use of thin-layer chromatography, and of these 37 no definite abnormal ganglioside patterns of any type were found in 25. During the same period 11 cases of Tay-Sachs disease have been studied.

Nine of the 37 cases studied since 1962 showing abnormal ganglioside patterns together with one other case are shown in Table 11. These cases are extremely interesting; the first was a patient who died in 1964 aged 32, having been described clinically in some detail by Glasgow in 1957. Unfortunately, no cerebral tissue was examined during life, and the initial studies on this patient were made five years before our use of thin-layer chromatography. Fortunately she lived until 1964 and at autopsy brain and various organs were examined, by which time total cortical gangliosides were considerably diminished. During life the bone marrow was examined and showed 'foam cells'. Thin-layer chromatography of lipid extracts of cerebral cortex, liver and spleen all showed the typical picture of G_{M1} gangliosidosis as described by Landing *et al.* (1964). The second and third cases were studied at biopsy and autopsy respectively, the third case also demonstrating a similar bone marrow picture. The fourth patient, a child of 11 months with lesions in various viscera as well as the brain, showed a typical ganglioside picture on thin-layer chromatography with a marked increase in G_{M1} (*see* Fig 5). In this case Dr R B Ellis of the Institute of Child Health examined a small portion of brain and demonstrated a reduction of β-D-galactosidase to 30% of normal.

Two brothers (K P and P P) classed as atypical are included showing normal ganglioside levels and lesser amounts of G_{M1} than the preceding cases, but with raised levels of G_{M3} and G_{M4} not seen in the other four cases. It must be stressed that the viscera all showed abnormal ganglioside patterns, similar to those seen in the brain.

There have been 3 cases (C G, L F and A M) in whom there was a different ganglioside abnormality, G_{M1} being reduced in amount and G_{D1a} increased. This is illustrated in Fig 6, but unfortunately the enzyme involved is not yet known; however, its possible site is indicated in Fig 7.

Finally there was one case of G_{M2} gangliosidosis of late onset (Type 3) in a boy of 5 (J N)

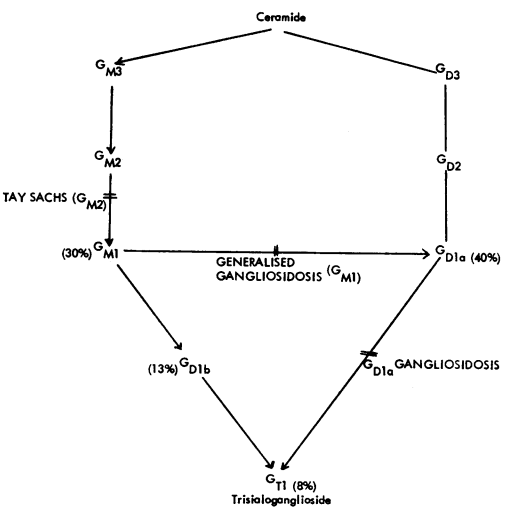

Fig 7 *Metabolic pathways of ganglioside to show sites of enzymes involved in Tay-Sachs disease and amaurotic familial idiocy. (Figures in brackets indicate amounts present normally)*

Table 12
Estimated amounts of each ganglioside in different types of disease (as percentage of total NANA)

		G_{M1} *Gangliosidosis*			
	Tay-Sachs				
Ganglioside	G_{M2}●	*Typical*	*Atypical*	G_{D1a}	■*Normal*
G_{M4}	–	4	12	6	–
G_{M3}	–	4	18	11	1
G_{M2}	82·5	–	–	–	3
G_{M1}	5·4	75	37	20	30
G_{D1a}	7·8	14	19	44	40
G_{D1b}	4·2	3	14	19	13

●Wherrett & Cumings (1963)
■Cumings *et al.* (1971)

who was still alive three years later. Estimated amounts of ganglioside in each fraction in some of these cases are seen in Table 12, confirming the macroscopic inspection of the thin-layer chromatogram.

I must comment briefly concerning the problem of the classification of some of these conditions. In general, the term sphingolipidosis can be applied to six disorders affecting the brain, for in all of them sphingosine is a component part of the lipid stored or present in excess in the tissues. Four of them, Gaucher, Niemann-Pick, Krabbe and metachromatic leukodystrophy, present few problems. In the other two conditions, Tay-Sachs and amaurotic familial idiocy, it is possible on a biochemical and histological basis to separate these into a number of subgroups. There are now known to be three types of Tay-Sachs disorder depending upon the enzyme affected, even though all show a raised G_{M2} ganglioside level as well as the characteristic EM appearance. Amongst the group of conditions called amaurotic familial idiocy there is firstly the variety described by Zeman as ceroid-lipofuscinosis with no abnormality in quantity or type of ganglioside but with a characteristic histological picture. This corresponds to the so-called Batten type. Secondly, there is the Landing *et al.* type of neurovisceral lipidosis with an increase of G_{M1} and with a known enzymic abnormality. Finally, there may be a group in which there is an increase of G_{D1a} of which we have seen three cases, and in which the actual enzyme involved is not yet identified. There is thus a large field among the remaining types of amaurotic familial idiocy which must be delineated more accurately before we can arrive at an exact classification of all the clinical varieties of this disorder, and it is hoped that some stimulus will have been given for further research in this and related metabolic diseases.

Acknowledgments: My sincere thanks are given to the many clinicians and pathologists who so willingly allowed me to examine patients and specimens, to all my laboratory staff, technical and secretarial, and to the Department of Medical Illustration of the National Hospital for their expert assistance.

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