1-carboxy-lyase, EC 4.1.1.15). N. A. Small & J. B. Holton (unpublished work) investigated the effect of histidine metabolites on the above enzymes in brain homogenates. Inhibition of both enzymes was demonstrated, particularly with imidazolylpyruvic acid, but these were generally lower than with the corresponding phenyl derivatives.

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Gangliosidoses

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An inherited disease with storage of an unknown glycolipid, Tay-Sachs disease, gave the impetus to the first systematic study of the chemistry of gangliosides (Klenk, 1942). They are a family of glycolipids mainly confined to the nervous tissue of vertebrates. In mammalian brain the parent ganglioside (G_{M1}) is a monosialosyl-*N*-tetraglycosylceramide:

to which can be further attached one to three sialic acid groups to the terminal galactose or to another sialic acid. The only sialic acid detected in brain

gangliosides is *N*-acetylneuraminic acid. Besides these major gangliosides, which constitute more than 90% of the normal mammalian brain gangliosides, there occur also species lacking the terminal galactosyl or galactosyl-*N*-acetylgalactosaminyl units. The latter were reported to be intermediary products in the biosynthesis and biodegradation of gangliosides (Svennerholm, 1966). About the same time Kaufman, Basu & Roseman (1966) reported that the major monosialoganglioside (G_{M1}) was formed by a stepwise biosynthesis (starting with lactosylceramide) in which each step was catalysed by a specific transferase. This pathway has now been extended to the start with ceramide (Basu, Kaufman & Roseman, 1968). Gatt and associates (cf. Leibovitz & Gatt, 1968) at the same time purified enzymes that catalysed the degradation of ganglioside G_{M1} in the opposite direction to its biosynthesis. The ganglioside transferases seem to have a high degree of specificity, although they are still incompletely known.

From a theoretical point of view an inherited disorder of ganglioside metabolism, a gangliosidosis, can be assumed for each step in the biosynthesis and biodegradation of the gangliosides. An indication of such a disturbance would then be a primary accumulation of a specific ganglioside or neutral glycolipids in the tissue. Table 1 depicts the stepwise biosynthesis and degradation of the gangliosides and the known disorders in which a storage of a glycolipid occurs.

During the last few years intensive studies have been devoted to the search for the enzymic lesion. There is still no evidence for primary defects in the ganglioside transferases, but a deficiency in the degradative enzymes has been shown to occur in two disorders. In the generalized gangliosidosis (or G_{M1} -gangliosidosis) Okada & O'Brien (1968) demonstrated a profound deficiency of β -galactosidase activity with both *p*-nitrophenyl-2-D-galactopyranoside and labelled ganglioside G_{M1} as substrate. The deficiency of β -galactosidase was demonstrated in all tissues investigated, which is to be expected for the primary defect in an inherited disease.

In Tay-Sachs disease (G_{M2} -gangliosidosis) there is no generalized diminution of β -*N*-acetylgalactosaminidase activity, but no liberation of *N*-acetylgalactosamine could be detected when G_{M2} -

ganglioside or its corresponding asialoderivative was used as substrate. The final proof of an enzymic deficiency of ganglioside- β -*N*-acetylgalactos-

Table 1. *Pathway for the biosynthesis and degradation of brain gangliosides.*

Abbreviations: Cer, ceramide; Glc, glucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; NAN, *N*-acetylneuraminic acid.

Biosynthesis		Biodegradation	Glycolipid	Inherited disorder
Glycolipid glucosyl-transferase	Ceramide ↓ Glc → Cer	Glycolipid β-glucosidase		
Glycolipid galactosyl-transferase	Gal → Glc → Cer	Glycolipid β-galactosidase	Glycosylceramide (glucocerebroside)	Gaucher disease (infantile form)
Glycolipid sialyl-transferase	Gal → Glc → Cer	Glycolipid neuraminidase	Lactosylceramide	Neurovisceral gangliosidosis (G _{M3} -gangliosidosis)
Glycolipid <i>N</i> -acetylgalactosaminyl-transferase	↑ NAN Gal → Glc → Cer	Glycolipid β-hexosaminidase	Monosialosyl-lactosylceramide ganglioside G _{M3} (hematoside)	
Glycolipid galactosyl-transferase	GalNAc → Gal → Glc → Cer		Monosialosyltri-glycosylceramide ganglioside G _{M2}	Tay-Sachs disease (G _{M2} -gangliosidosis)
	↑ NAN	Glycolipid β-galactosidase	Monosialosyltetra-glycosylceramide ganglioside G _{M1}	Generalized gangliosidosis (G _{M1} -gangliosidosis)
	Gal → GalNAc → Gal → Glc → Cer			
	↑ NAN			

aminidase activity can only be obtained by using a labelled ganglioside, which has not yet been prepared. Another possibility for the accumulation of G_{M2}-gangliosides could be a deficiency of glycolipid galactosyltransferase. However, the incorporation of labelled galactose in the form of UDP[¹⁴C]-galactose was slightly higher in brain tissue from patients with Tay-Sachs disease than in age-matched controls.

It has not been possible as yet to study the enzymic defect in G_{M3}-gangliosidosis, because only one case has been reported (Jørgensen, Blackstad, Harmark & Steen, 1964; Pilz, Sandhoff & Jatzkewitz, 1966) and all the material was fixed in formalin. A deficiency in a glycolipid β-galactosidase different from that affected in G_{M1}-gangliosidosis can be assumed.

In Gaucher disease, Brady, Kanfer & Shapiro (1965) demonstrated a nearly total lack of glycolipid β-glucosidase activity in an infantile case and a severe deficiency in adult cases. In brain tissue from children who had died from the infantile form of Gaucher disease no β-glucosidase activity with glucosylceramide as substrate was found. It was also demonstrated that glucosylceramide was stored in brain and that it had the fatty acid and sphingosine composition that is characteristic of

brain gangliosides (Svennerholm, 1966). Thus, the infantile form of Gaucher disease may be classified as a gangliosidosis. In the juvenile form there is no indication of any disturbance of the brain ganglioside metabolism, although the glycolipid β-glucosidase activity is very low.

All the four above-mentioned diseases are probably caused by a mutation of a structural gene. In a fifth gangliosidosis, Hurler disease or gargoylism, a mutation of a regulatory gene can be assumed. In Hurler disease gangliosides are accumulated in the ganglion cells but the increase is not limited to a specific ganglioside (Leeden, Salsman, Gonatas & Taghavy, 1965). The storage of brain gangliosides is combined with a serious disturbance in the metabolism of acidic glycosaminoglycans, which are stored in mesenchymal tissues and excreted in large amounts in the urine. The biosynthesis of sialic acid is regulated by a control mechanism that is closely allied to hexosamine formation: the formation of glucosamine 6-phosphate from glucose 6-phosphate is inhibited by UDP-*N*-acetylglucosamine and the formation of *N*-acetylmannosamine (precursor to *N*-acetylneuraminic acid) from UDP-*N*-acetylglucosamine is inhibited by CMP-NAN (Kornfeld, Kornfeld, Neufeld & O'Brien, 1964). A mutation of the

regulatory gene for the hexosamine formation might lead to increased biosynthesis of both glycosaminoglycans and gangliosides.

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Wilson's Disease

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Wilson's disease: the problem. Also known as hepatolenticular degeneration, this is an hereditary metabolic disease associated with abnormal storage of copper in the liver, brain and kidneys. The pathological changes commonly found in these organs have been attributed to copper toxicity. The disease is inherited in an autosomal recessive fashion; a gene frequency of between 1/200 and 1/500 has been calculated. Symptoms may appear at any age from 6 to 40 years with a maximum incidence around 13 years. In children before puberty the illness usually appears as liver damage, and in some cases death may occur before the brain is involved. In older age groups the brain usually bears the brunt of the illness, the motor systems only being involved, even though copper is deposited in equally high concentrations in the sensory areas. Copper is also deposited in the kidneys, leading to renal-tubular defects, and in the cornea, giving rise to the typical pigmented rings. The disease, untreated, runs an almost invariably fatal course lasting for a few weeks to a few years at most.

Normal handling of copper. The normal intake of copper in the diet is between 2 and 4 mg. daily. As very little copper is excreted in the urine, balance must be maintained either by a barrier to absorption or by efficient biliary excretion. Studies using radioactive copper suggest that the latter is the mechanism normally employed, at least as far as

ionic copper is concerned. Within 10 min. of giving ^{64}Cu by mouth it can be detected in the plasma, and at about the same time surface counters will detect the appearance of radioactivity in the liver. In normal individuals between 60 and 80% of an injected dose of ^{64}Cu is present in the liver within 24 hr. The radioactivity in the blood shortly after oral or intravenous administration is present both in the cells and in the plasma, where it is bound mainly to albumin and also in small amounts of amino acids. Albumin-bound copper is given up to the liver, where some of it is incorporated into the serum protein caeruloplasmin before being returned to the circulation (Bearn & Kunkel, 1954). Caeruloplasmin is an α -globulin of mol.wt. 160 000 (Kasper & Deutsch, 1963), each molecule containing 8 atoms of copper, which are present as cuprous-cupric pairs, the valence-changing ions forming a common acceptor site able to activate and reduce an oxygen molecule (Broman, 1964): the physiological substrate of this oxidase, if any, is unknown. Studies with caeruloplasmin labelled with ^{64}Cu have shown that there is no exchange *in vivo*, so that this is probably not a transport protein for the metal (Sternlieb, Morell, Tucker, Greene & Scheinberg, 1961). Copper is carried to the tissues as either an albumin or an amino acid complex. It has been calculated that the normal adult male contains in his body about 100–150 mg. of copper, present as a series of copper proteins, hepatocuprein, cerebrocuprein, erythrocuprein and two with enzyme activity, tyrosinase and cytochrome *c* oxidase. As the latter enzyme is present in most if not all cells of the body as the terminal step in the electron-transfer chain, copper is clearly essential for life.

Copper: the biochemical lesion. An experimental model of Wilson's disease in animals is not available, but it has been possible to show that the intracerebral injection of Cu^{2+} rapidly leads to convulsions and death. (Peters & Walshe, 1966). Protection against convulsions was given by the synchronous injection of one of the thiols 2-mercaptopropan-1-ol, thioctic acid or penicillamine. Studies *in vitro* showed that Cu^{2+} blocked pyruvate oxidation by mitochondria; more organized tissue breis or slices showed no significant inhibition of oxygen uptake at Cu^{2+} concentrations that induced convulsions *in vivo*. Further experiments showed that Cu^{2+} also blocked the Mg^{2+} - Na^{+} - K^{+} -activated adenosine triphosphatase of pigeon brain microsomes, and it was suggested that this led to the onset of convulsions *in vivo* (Peters, Shorthouse & Walshe, 1966).

Enzymic studies have not been possible on fresh brain from patients with Wilson's disease but it has long been known that copper concentrations are very high in brain, liver and kidney. In the plasma there is a deficiency of a caeruloplasmin, leading to a