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Significance of Plasma Glycolipid Levels in Normals and in 3 Disorders of Brain Glycolipids.* (26577)

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In 2 neurological disorders of myelin, the kidney may be involved as well as the brain. In the metachromatic form of diffuse cerebral sclerosis, sulfated glycolipids are increased in cerebral white matter, kidney tubules, and urine sediment(1 - 3). In one recent case of the globoid form of diffuse cerebral sclerosis, some kidney epithelial cells have contained unusual material, the staining of which resembles that seen in the diseased white matter(3). Sugar determinations in a heterogeneous lipid extract from one earlier case of this globoid form suggested the possibility that glycolipids might be increased in the brain(4). Recent chemical and direct experimental evidence has suggested the possibility that it may be glycolipids more in the "true" cerebroside category which increase locally in certain globoid cases(3,5). It is the cerebron-like "true" cerebrosides which cause a globoid-like response when injected into rat white matter, whereas thus far injections of other glycolipids (such as sulfatides or gangliosides) have not caused this response (3,5).

If organs as remote as brain and kidney are both involved in some type of glycolipid disorder, 2 major questions arise: (1) Does the material in the kidney merely reflect concentration by the kidney of that material from the diseased brain which has "overflowed" into the circulating plasma? (2) Or, is the material in the kidney largely independent of the plasma and simply an expression in the kidney of the same type of metabolic lesion which occurs, separately, in the brain? One important differentiating point would be the plasma glycolipid level.

The following initial study of plasma glycolipids is reported here, both because these problems of lipid deposits are becoming of wider general biological and conceptual significance and because a most unusual opportunity was presented to study simultaneously a selected series of 4 living patients with 3

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rare genetically-determined diseases, the causes of which are still obscure(3).

Material and methods. Identifying information and biopsy findings on the 2 sibling patients with metachromatic leucoencephalopathy† and on the one patient with a globoid form of diffuse sclerosis (Krabbe's disease)‡ are published elsewhere(3,6-8). Nonhemolysed plasma from the normal male and female control children of comparable ages and from the abnormal control with gargoylism§ was drawn at comparable times. Plasmas were separated and promptly frozen at -30°C. Control samples were then analysed at the same time, and in the same series with the patient material. The method of Svennerholm(9) was used, with exceptions noted below. The primary extraction of the dried, lyophilized plasma was done in 4 stages with standardized stirring at room temperature(3). A (larger) total of 80 volumes of chloroform-methanol (2:1, v/v) was used per 10 ml of original plasma. After centrifugation at 4000 rpm for 20 minutes and filtration through sharkskin paper, this first lipid extract was dried in a vacuum dessicator. This lipid residue was then reextracted with a total of 16 ml. of solvent and was again filtered. It is emphasized here that water-soluble materials were then removed from this lipid extract by 4 separate washes as indicated (9), but performed in this instance with the aid of a stronger (730 mg %) NaCl solution. The proportion used for the first wash and for the equilibrated wash solution was one volume of NaCl solution to 5 volumes of chloroform-methanol solution. Lipid methods used here have been previously tested in our laboratory and are the same as those cited elsewhere(2). Two separate glycolipid determinations were made at separate times on each purified lipid extract and the results averaged. Deviation from the mean was

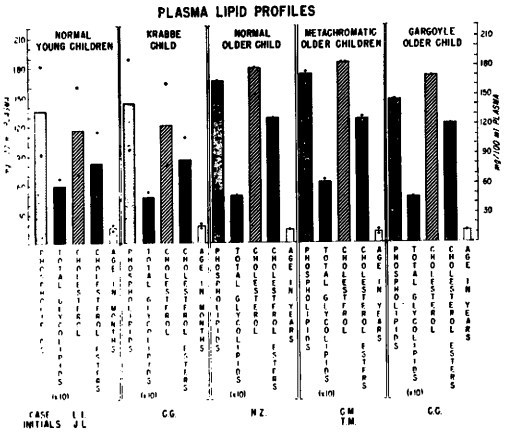

FIG. 1.

† 10.3%. All lipid values are expressed in mg per 100 ml of the original plasma before this plasma was diluted with citrate solution. In Fig. 1, the total glycolipid values have been multiplied by 10 so that they will stand out more easily for comparison among the other lipids in the profile. Dots stand for separate patients or separate determinations; bars stand for means.

Results. With the technic used in this study, glycolipid values for normal children, age 1-12 years, ranged from 4.9 to 6.5 mg %. In Fig. 1, results for normal young children (12-19 mo.) are separated from the normal older child (12 yr.) so that controls of appropriate age may be compared with the patient material.

In the 2 metachromatic patients, the plasma glycolipid values of 6 and 6.3 mg % were only slightly higher than in their control group of comparably older children (4.6-4.9 mg %). In view of the fact that these 2 metachromatic patients are siblings, it is of interest that they show a close correlation in their values, not only in their glycolipid results, but throughout the other determinations in the profile.

The young child with Krabbe's disease (globoid form) shows *no* elevation of plasma glycolipids (4.3-5.1 mg %). It is uncertain whether her somewhat lower values for other lipids at age 15 mo. are to be related to a period of several previous weeks of undernutrition and pneumonitis. This is because her controls show a similar range of vari-

† Age at onset and age at time of test: C.M. 18 mo. and 12 yr. (autopsy verified); T.M. 8 mo. and 9 yr.

‡ C. G. 4 mos. and 2 samples at 15 mo. and 18 mo. respectively.

§ C. C. 1 year and 12 yr. Classical, retarded male gargoyle without cloudy corneae.

ations, and because some serum lipids (*e.g.*: cholesterol) are known to show day to day fluctuations(16). A dilution artifact is not excluded.

Two separate samples of plasma were available from the 2 normal young children, from the one globoid child, and from the 2 metachromatic children. In each patient, the lower of each of these 2 glycolipid values happens to correlate with the lower of the 2 values for phospholipids, total cholesterol, or cholesterol esters, respectively.

Discussion. In the 2 older metachromatic patients, as the neurological and kidney disorder is far advanced, it is questionable whether their slightly higher plasma glycolipid values reflect a plasma trend of primary significance. For example, some general tendency has been noted for certain *non*-glycolipids in blood to be raised nonspecifically in children who have a wide variety of neurological diseases other than those studied here(10). In addition, glycolipid results in 3 younger metachromatic children have appeared since completion of the present study. In these patients, the glycolipid values (3.4-3.9 mg %) fell within the range of the normals (2.7-4.7 mg %)(13).

Even the presence of an early, gross and consistent elevation in a certain plasma glycolipid fraction would leave a problem unsettled. Specifically, such an elevation need not only reflect material actually enroute from degenerating brain; it might rather represent material first synthesized in the kidney (or some other systemic organ) which then spilled out into the plasma. However, a *normal* plasma glycolipid value may be one point more in favor of an independent origin of a metabolic abnormality in brain and kidney. Of particular interest in this regard is the normal trend in plasma glycolipids of the living globoid (Krabbe) child. Viewed together with the small absolute amounts of glycolipids which we have been able to extract from devastated white matter of 2 other globoid patients(3,5), these results would not suggest one disease which has a necessarily simple process of organ storage and plasma overflow. If such exists, it would not appear to be readily definable by these methods. Moreover, with plasma glycolipid values if anything slightly toward the low normal side, the potential mechanism in storage diseases of an increased binding of tissue glycolipids to *other* moieties in tissues still remains to be considered. Because the metachromatic and globoid categories of diseases may each include subtly different disease mechanisms(3,5), it follows that in future series of cases each patient must still be individualized.

This lack of elevation in plasma from CG is of further interest because of the recent report of an *increase* of plasma glycolipids in (splenectomized) children with Gaucher's disease—a disorder in which a glycolipid storage in visceral organs is established(11). Hence, the old issue of what role the plasma lipids play in the visceral deposits in an *individual* Gaucher patient is again reopened, because Thannhauser found *normal* glycolipid values in (non-splenectomized) adult Gaucher patients(12).

Nothing is known about the turnover rate of the plasma glycolipid compartment *per se*, or of the equilibrium it shares with various organ systems in health or disease. However, for the brains of infants and young children to grow rapidly, it is plausible that certain brain glycolipids are in a more dynamic relationship with their plasma precursors and plasma counterparts (at least when contrasted with the adult). Moreover, despite the fact that the brain and the plasma compartments are several steps removed metabolically, and are separated by a "blood-brain" barrier in the adult, there may still be a significant degree of *internal* turnover within these brain glycolipids themselves(14,15). A tendency in the present series for a lower glycolipid value to correlate with lower values for other lipids from the same patient is more likely fortuitous than a reflection of a real metabolic interrelationship.

Glycolipid results here, as well as those of Svennerholm, and the still lower values of Thannhauser, are lower than the "cerebroside" results reported earlier, presumably in part because any water-soluble glyco-complexes‖ (which might be extracted) would

‖ e.g.: mucopolysaccharides, gangliosides, etc.

now be removed during the washing steps(9). The classical gargoyle was originally included in this series, both as a possible test of this very point and as an appropriate control for the 2 authentic metachromatic patients who also have these gargoyle-like laboratory findings. However, in the first lipid extract of gargoyle plasma, a moderately turbid precipitate developed, some of which then adhered to the glassware. This precipitated material was no longer soluble at room temperature in a chloroform-methanol solution. Lipid results recorded for all patients here clearly refer to the usual extractable and continuously *soluble* lipids, and are not intended to include a less conventional plasma fraction which would be removed by precipitation and filtration (as was this gargoyle fraction, for example).

The small size and terminal condition of our patients limits the volume of plasma which can be safely withdrawn for analyses. Hence, here as elsewhere(13), there is a corresponding limitation on our knowledge of exactly how much of the total plasma glycolipids may exist in the form of sulfatides, and how much is in the form of *non*-sulfated glycolipids.

Summary. 1. Plasma glycolipids and other plasma lipids were studied in 2 diseases affecting the myelin glycolipids, in gargoylism, and in controls of appropriate age. 2. Plasma glycolipid levels in this series of normal children ranged from 4.9-6.5 mg %. Those in 2 metachromatic children ranged from 6.0-6.3 mg %. In the globoid child, plasma glycolipids ranged from 4.3-5.1 mg %. 3. Data obtained with these methods would not appear to establish a disorder of total plasma glycolipids *per se* as the primary source for systemic deposits in the patients studied. 4) The significance of the plasma glycolipid level is considered, and the need for individualization in further studies is stressed.

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