type of synthetic substrate should permit its estimation in blood and urine. Accurate measurement of the blood levels of proteolytic enzymes of pancreatic origin has not been achieved. The presence of inhibitors, enzymes of similar properties and specificities, and complicated activation systems have raised doubts as to the reliability of these methods. Attempts to measure carboxypeptidase in serum and urine are in progress and preliminary studies suggest that this is possible.

Summary. 1. A method is described for determination of carboxypeptidase in human pancreatic juice. Eighteen samples of pancreatic juice gave an average value for active carboxypeptidase of 3.9 mg % with a range of 2.2-6.2 mg %. Total enzyme concentration representing active enzyme plus procarboxypeptidase gave an average value of 52.8 mg % with a range of 28.3-134.0 mg %. 2. A significant amount of active carboxypeptidase was found in fresh pancreatic juice. High values of active and total carboxypeptidase were found initially postoperatively but these declined on about the third postoperative day. 3. Carboxypeptidase was found to be more stable to autolysis than trypsin.

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Metachromatic Sulfatides in Cerebral White Matter and Kidney.* (24627)

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Of considerable biological interest is the striking metachromasia of normal myelin with toluidine blue(1). Color change of this blue dye toward red is consistent with the presence of compounds rich in negatively-charged acid groups(6). While acid mucopolysaccharides are commonly associated with metachromatic staining, it is less fully appreciated that acidic lipids may also cause intense metachromasia. These strongly charged anionic groups might be expected to bind cations. Such a process might be relevant to bioelectric potentials involved in the nerve impulse and to rapid movements of K+, Na+, Ca++, etc. across membrane systems in other body tissues.

In previous staining experiments, made *in vitro* on naturally-occurring sulfatides, it was shown that at least part of the normal lipid metachromasia of white matter may be related to sulfuric acid ester groups(2,7).

It was thus of interest to extend these staining observations to a measurement of lipid sulfuric ester groups in 2 tissues where cation transfer is biologically prominent. In the present study normal, metachromatic white matter was contrasted with normal kidney, where lipid metachromasia is negligible. Pertinent diseases which afford a range of sharply contrasting values were also included. Results bear ultimately on the intriguing function of myelin and other sulfatides in health and disease.

Materials and methods. Applicability of partitioning, separation, and identification(3, 4,8,9,11) methods used in this study were previously verified(2,7), (Witmer, F., and Austin, J. to be published). Histologically nor-

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mal material from 7 human brains and 7 kidneys was then analyzed by identical technic with contrasting material from 6 mildly abnormal brains, 3 severely abnormal brains, and 3 severely abnormal kidneys.† Cerebral white matter was from centrum semiovale. Representative and comparable ages were used, preference given to the younger age group after myelination.‡ Normal and abnormal cases were matched as to location of white matter sample and length of fixation. Formalin fortunately maintains cerebrosides (10) and, it would appear, their sulfuric esters, or sulfatides, as well. Kidneys were capsule free. Formalin-fixed organs were homogenized, dialyzed 48 hours against water, lyophilized and dried to constant weight in a vacuum desiccator over fresh P_2O_5. Lipids were extracted from 0.5-2 g of dry powder with large volumes of chloroform-methanol mixtures. Red metachromatic lipids were traced by filter paper spot test(2) through each subsequent separation. An aqueous sodium chloride wash (730 mg %) removed water soluble materials(4). An isolation procedure was then used, reported by Lees(3) to yield a sulfatide with the chemical composition of cerebron sulfate, (referred to here as cerebroside sulfuric acid ester). Careful standardization of proportions in this procedure concentrated metachromatic lipids into one final fraction. Here they were precipitated twice with KCl in dilute methanol, and dried with N_2, P_2O_5 and vacuum. Sulfuric acid ester groups ($-OSO_3$) in final dry sulfatide fractions were next determined by infra-

† Included in the last 2 categories are 3 brains and 3 kidneys from 4 patients with metachromatic leucoencephalopathy (ML) (#), of special interest because increased amounts of metachromatic lipids are seen histochemically in white matter and kidney tubules (2,6,7).

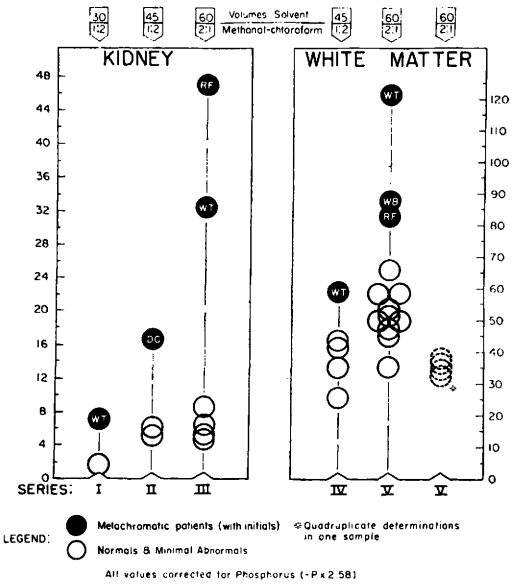
W. T. age 9; D.C. age 3½, (Western Reserve Hosp.); W. B. age 3½, (Mass. Gen. Hosp.); R. F. age 5, (Baylor Hosp.). (See also references 2, 6, 7.)

‡ Median age white matter from normal, mildly abnormal, and metachromatic leucoencephalopathy (M) respectively: 7, 6, 5 years; mean age 10, 15, 5 years; range 1½ - 70, 6 - 34, 3½ - 9 years. Median age kidney from normal and ML respectively: 6, 5 years; mean age 9, 6 years; range 1½ - 34, 3½ - 9 years.

TABLE I.

CEREBROSIDE SULFURIC ESTERS

mg. per Gram (dry weight)



red spectrophotometry at their absorption peak (8.02 μ). By double-beam infrared analysis the sulfatide isolated by this method from unfixed kidney (I, case W.T.) was spectroscopically identical both qualitatively and quantitatively with a purified reference sample of cerebroside sulfuric ester. This sample (kindly furnished by Dr. S. J. Thannhauser) was prepared by another method(5) and its spectrum is confirmatory of Lees' report(3). For purposes of convenience, lipid sulfuric acid ester (SAE) absorptions are therefore corrected by comparison with and expressed in terms of this standard cerebroside sulfuric acid ester of Thannhauser. Perkin-Elmer spectrophotometers were used with densitometer measurement, corrected for blank, of KBr discs on model 112, double beam technics on model 21, and Sartorius-Wercke microbalance weighing . Nominal corrections were made for phosphoric acid esters(5) by subtracting P x 2.58(9).

Results. The last 5 series of analyses contain 32 determinations. Each series is plotted separately and vertically in Table I, (I-V).

The lowest 4 values for white matter in Series IV and lowest 6 in Series V range from 25-44 mg/g and 35-51 mg/g respectively. The

only "normal" patients among these lower values are those at the extremes of age (15 months, 17 months, 70 years); the rest are mildly abnormal brains§ (○). In series V, in the next cluster from 53-65 mg/g are healthy normals of intermediate ages 4-20. Values in ML here (●) are 127-185% those of the highest normal white matter value ($p<.05$ and $p<.005$ by extreme means test for whole series).

Normal kidneys show small amounts of SAE. Averages are 5.4-6.0 mg/g in Series II and III. These values are only 1/10 that of normal mature white matter. In strongly metachromatic kidneys from ML patients, average elevation of SAE is 3-6 times average normal (III $p<.005$). Conspicuous increases in ML cases are seen grossly by their stronger metachromasia and heavier test tube precipitation. Single-beam qualitative infrared analysis shows no essential difference between comparable normal, abnormal, ML, and reference standard sulfatides nor do double-beam high resolution technics in representative samples tested.

Discussion. The sulfatides extracted correspond histochemically, in double refraction, and in solubilities both with the diffuse lipid metachromasia of normal myelin and with excess material in brain and kidney in ML(2,6, 7).

Slightly lower than average values in normal infancy may reflect chemical immaturity of myelin with its SAE synthesis still incomplete. Reduction in old age might indicate a relative preponderance of myelin catabolism. As was found, slightly lower SAE values would also be expected in diseases with mild abnormalities or actual loss of white matter substance.

In ML, on the other hand, a paradox exists. Here, *above* normal amounts of SAE occur in brains which not only lack normal myelin, but are also lacking in "neutral fat" stages which would indicate previous myelin breakdown (7). It is likely that such a gross excess of cerebroside SAE would be a crucial departure from the normal sequences of cerebroside metabolism. In the slow but still dynamic metabolic turnover of myelin, a subtle error affecting synthesis or resynthesis may ultimately lead to inadequate myelin formation. Thus, the excess SAE found may help reconcile the apparent paradox. It is consistent with the possibility that in ML there may occur more of a gradual failure to properly build up and renew "normal myelin" in the first place, and perhaps for this reason, then, less of an apparent *de*myelination through conventional pathways.

Infrared studies contribute no evidence for the possibility that *gross* structural changes in the sulfatide unit itself might differentiate the normal and abnormal conditions studied. It will be noted that the SAE excess appears, descriptively, to constitute a "sulfatide lipidosis" which is similar to the accumulation of other naturally-occurring lipids in "lipid storage" disorders(13).

It is plausible that much higher SAE values in normal white matter, 10 times those of kidney, may reflect a relatively more important biological role for sulfatides in the nervous system.

Discussion of factors which influence phosphorus in sulfatide fractions(3) is outside the scope of this paper. Sulfatides are expressed in keeping with the existing (1955) formula for cerebroside SAE which has no P(5), but provision is made here for other formulae by recording % P found. (Range kidneys: 0.70-3.6% P in normals; 0.56-0.75% P in M.L. Range white matter: 1.27-2.38% P in normals and minimal abnormals; 0.19-0.47% P in M.L.)

Because of improvement in yields from Series I to V with technical improvements during the past 2 years, values are to be compared within each series. Extraction with greater proportions of methanol and more solvents (top Table I) gives higher yields. Duplicate determinations on duplicate samples show reasonable agreement (13%). The sparse literature on sulfatides has no strictly comparable data. If the sole normal sulfolipid value for adult mammalian white matter

§ IV: Sudanophilic Diffuse Sclerosis, Familial Leucoencephalopathy (Minimal); V: Chronic Hydrocephalus, Familial Polioencephalopathy (2 separate cases), Multiple Sclerosis.

is corrected (x 3.3) to dry weight one figure is 39 mg/g(12). This agrees with normal SAE values obtained by more sensitive methods in the present series. It is clear that many other lipid analyses, in common with these data, are to be regarded as first-order approximations, of greater value for comparison within one controlled series than as absolute numbers. Neither this nor other current procedures neatly separate sulfatides quantitatively from nonsulfatide fractions. This factor was minimized by 1) the significant number and selected variety of simultaneously analyzed samples, 2) recording corrections made for phosphorus content, and 3) the fact that metachromatic lipids which enter the non-sulfatide fraction do so to a greater degree in ML cases where they are clearly increased in the lipid extract to begin with. Consistent biochemical, microscopic, spot test, and gross visual correlations among brains, kidneys, normals, mildly abnormals, and metachromatic cases, reproduced even with different extraction mixtures, support the coherence of the data.

Summary. 1) Metachromatic sulfuric acid esters (SAE) were measured in sulfatide fractions extracted from normal and abnormal white matter and kidney. 2) Slightly lower than average SAE values for white matter were found in early childhood, in old age, and in diseases interfering with myelin metabolism. 3) The average SAE value in normal mature white matter was 58 mg/g. This was 10 times the value for normal kidney and corresponds with the much greater lipid metachromasia in white matter histologically. 4) Statistically significant elevations in sulfatide values were found in white matter and kidney in one disease (metachromatic leucoencephalopathy). 5) This accumulation may be related to an error in sulfatide metabolism, and appears, descriptively, to constitute a "sulfatide lipidosis."

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In vivo Measurement of Radiophosphorus and Radiostrontium Absorption in Rats.* (24628)

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Hamilton(1) demonstrated *in vivo* the absorption of orally administered radioisotopes of Cl, Br, I, Na and K in humans. In the following experiments, this method has been modified to measure absorption of radiophosphorus and radiostrontium in normal adult rats. The isotopes are given by stomach tube, and the radioactivity which accumulates in the

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