TABLE I.

Name	Particle size in μ
Linde silica	.02–.05
Columbia-Southern Hi-Sil 101	.03
Dow Corning silica (unpelletized)	.01–.02
Powdered silica gel	
Sodium metasilicate	
Carborundum Co. Fibrofrax (silica fibers)	

humans.

Summary. Powdered silica or Fibrofrax mixed with the rat diet to the extent of 10%, produces no tissue pathology in the gastrointestinal tract, liver, spleen, pancreas, adrenals, lung or mesenteric lymph nodes when fed continuously for 3 months.

We are indebted to Columbia-Southern Corp., Dow-Corning Corp., Carborundum Co. and Dr. Charles P. Carpenter for generous supplies of the silica.

Received November 20, 1957. P.S.E.B.M., 1958, v97.

Cerebrospinal Fluid Enzymes in Central Nervous System Lipidoses* (with particular reference to Amaurotic Family Idiocy) (23733)

STANLEY M. ARONSON, ABRAHAM SAIFER, GUTA PERLE AND BRUNO W. VOLK
Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

The diagnostic usefulness of certain enzyme levels in cerebrospinal fluid (CSF) and serum derived from cases of central nervous system (CNS) disease has recently been explored (1-5). Various authors have demonstrated transient glutamic oxalacetic transaminase (GOT) elevation in CSF derived from clinically determined or experimentally induced cerebral infarction(1,2). Other investigators, in evaluating the utility of CSF GOT in a broad spectrum of neurologic disorders, were unable to report any conclusive alterations in the levels of this enzyme(4).

Significant rises in serum GOT and aldolase have recently been recorded in cases of Infantile Amaurotic Family Idiocy (IAFI) (5). These findings indicating that a number of determinable serum enzymes is increased in one of the CNS lipidoses led to an assessment of their activity within the CSF of cases in this disease category. Concurrent serum levels were also determined to clarify any possible correlation between the two biological fluids. The activity of lactic dehydrogenase (LD) was also measured in the serum and CSF of selected lipidosis and control cases.

Material and methods. a.) Clinical: Repeated serum and CSF specimens from 9 patients with IAFI (at varying stages of the illness) and 2 cases of Niemann-Pick's Disease (NPD) with CNS involvement were studied in regard to GOT, aldolase and LD levels. Similar enzyme studies were performed on 33 additional children afflicted with other neurological disorders. b.) Experimental: 10 mongrel dogs, each weighing about 13 kg, were injected intramuscularly with 1 g (GOT enzymatic activity: 80,000 u./g) of fresh heterologous canine grey matter. An additional 3 dogs were inoculated with a similar homogenate previously incubated at 100°C for 1½ hours (GOT enzymatic activity, 0.0 u./g). All dogs were then sequentially studied by daily serum GOT determinations for periods up to 8 days. The method employed for determination of serum and CSF GOT was that of Karmen, Wróblewski and LaDue(6) based on spectrophotometric measurement in the near ultraviolet with the Beckman D.U. spectrophotometer. Serum and CSF aldolase activity was estimated with the colorimetric procedure of Sibley and Lehninger(7). The LD activity of the serum and CSF samples were also determined spectrophotometrically at 340 mμ with the Beckman D.U. using the method of Wróblewski and LaDue(8).

* This project was performed under auspices of Nat. Tay-Sachs Assn.

TABLE I. Enzyme Data in Serum and CSF from Cases of CNS Lipidosis and Other Neurologic Disorders.

Diagnosis	No. of patients	Age	Serum GOT, units/ml		Serum Aldolase, units/ml		Serum LD, units/ml	
Amaurotic family idiocy	9	9–14 mo*	127 (2)†	123–130‡	10.1 (2)	7.7–12.4		
		15–24 " *	92 (17)	55–152	8.9 (17)	5.4–15.7	1641 (4)	1362–1942
		25+ " *	65 (5)	41– 91	9.2 (5)	5.8–12.8	1599 (2)	1458–1740
Niemann-Pick's disease	2	10–22 "	170 (8)	91–248	9.3 (8)	6.9–12.5	576 (2)	504– 648
Cerebral palsy	15	13 mo–12 yr	39 (20)	20– 64	9.1 (20)	2.8–15.6	700 (3)	552– 870
C.N.S. anomaly	13	1–9 yr	36 (19)	20– 51	7.1 (19)	4.0–11.5	719 (2)	714– 724
Obstructive hydrocephalus	4	9 mo–9 yr	45 (2)	44– 46	15.1 (2)	13.9–16.5		
Craniopharyngioma	1	4 yr	50 (3)	37– 58	10.2 (3)	6.6–15.2		
Normal range				22– 40		5–10		200– 680
			CSF GOT		CSF Aldolase		CSF LD	
Amaurotic family idiocy	9	9–14 mo*	72 (2)	50–94	3.6 (2)			
		15–24 " *	38 (17)	22–60	1.4 (17)	.3–2.7	143 (4)	130–152
		25+ " *	33 (5)	24–47	.9 (5)	.1–2.2	176 (2)	140–212
Niemann-Pick's disease	2	10–22 "	32 (4)	25–41	4.6 (4)	2.3–7.4	75 (1)	
Cerebral palsy	15	13 mo–12 yr	10 (20)	5–18	.4 (20)	.1–1.0	25 (3)	20– 33
C.N.S. anomaly	13	1–9 yr	11 (19)	4–19	.5 (19)	.0–1.1	41 (2)	39– 43
Obstructive hydrocephalus	4	9 mo–9 yr	33 (5)	24–41	2.2 (4)	1.1–5.0		
Craniopharyngioma	1	4 yr	19 (3)	12–34	1.1 (3)	1.0–1.3		
Normal range				4–14		0–1		5–40

* Criteria for phases of disease given elsewhere(9).
† Arithmetic mean and, in parentheses, No. of separate determinations.
‡ Range of determined values.

Results. The serum and CSF GOT were invariably elevated in all cases of IAFI studied (Table I). The enzyme levels were higher in the initial phases of the disease. With further progress, the abnormal values diminished, but even in the protracted aspect of the disorder (beyond 2 years of illness), the average serum and CSF GOT values were well above the upper limits of normal. A distinct correlation between the simultaneously determined levels in the 2 biologic fluids was apparent. The serum and CSF GOT were considerably increased in NPD. Only one patient in the control group showed any consistent serum GOT rise (an 8 months old male child with severe progressive cerebral disease of unknown etiology). The same infant also showed an elevated CSF GOT. Three children, all with continuing obstructive hydrocephalus, demonstrated normal serum GOT but consistent elevations of CSF GOT. The CSF GOT in 1 case of confirmed craniopharyngioma was increased.

CSF aldolase was usually elevated in the 11 cases of CNS lipidosis (IAFI and NPD). A comparable increase was also noted in the children with hydrocephalus described above. The corresponding serum aldolase values in all groups were within the normal range.

LD levels (both in serum and CSF) were notably increased in the lipidoses, while they were approximately normal in the limited number of control cases studied (Table I).

In the dogs inoculated intramuscularly with grey matter emulsion, an approximately 2-fold rise in serum GOT was seen in the 2 days subsequent to injection. No such rise was noted in dogs injected with an inactivated cerebral homogenate or mesenteric fat administered in similar volume (Table II).

Discussion. It has been reported previously that serum GOT and aldolase are increased in cases of IAFI and NPD(5). The transient rise in serum aldolase was thought to be caused principally by the coexistent neuromuscular atrophy. Since myelopathic muscle atrophy has not been equated with any rise in serum GOT(4) and since no structural abnormality other than ganglionic necrosis has been identified in IAFI, it was provision-

TABLE II. Serial Serum—GO Transaminase Values (Units/ml) in 13 Dogs following Intramuscular Injection of Fresh or Heat-Inactivated Brain Grey Matter.

Inoculum	No. of dogs	Fasting before inoc.	Time after intramusc. inoc. 5 hr	1 day	2 days	3 days	4 days	7 days
Grey matter, 1 g	10	35 (20)*	55 (10)	64 (17)	62 (14)	44 (10)	39 (10)	38 (10)
		27–44 †	44–74	40–107	28–166	28–68	18–70	28–67
Heat inactivated grey matter, 1 g	3	31 (6)	35 (3)	30 (6)	39 (4)	27 (3)	30 (3)	31 (3)
		24–41	32–40	20–53	25–64	22–35	26–35	30–32

* Arithmetic mean and, in parentheses, No. of separate determinations.
† Range of determined values.

ally suggested that the sustained hypertransaminasemia was attributable to degeneration of brain, a tissue containing high concentrations of GOT. Significant quantities of CSF GOT were shown in all cases of CNS lipidosis regardless of disease stage, presumably due to enzymatic liberation. This suggests that the GOT elevations in both CSF and serum were the result of a single source of enzyme release. An appreciable but short-lived rise in serum GOT was demonstrated in dogs injected with fresh grey matter emulsion.

It would seem, therefore, that any disease process resulting in cerebral necrobiosis can cause an increase in CSF GOT levels, and if sufficiently pronounced, a rise in serum GOT content. The resultant enzyme elevation is also a guide to any prolongation of necrosis. Thus, if the brain damage is episodic (*i.e.*, cerebral vascular disease), the GOT rise will be transient. If, on the other hand, the nature of the underlying disorder causes a continuing breakdown of neural tissue, such as in CNS lipidoses(9), the secondary GOT elevation will be more enduring. Paranatal brain injury does not appear to cause any rise in CSF GOT, if the determinations are carried out years after the sustained damage. On the other hand, rapidly developing and progressive hydrocephalus with cerebral degeneration has resulted in a rise of CSF GOT.

Some have postulated that the blood-brain barrier causes a failure of correlation between serum and CSF GOT. Cases of hepatic diseases with hypertransaminasemia and normal CSF GOT levels are offered as supportive evidence(2). The close correlation of GOT enzyme content in the two fluids in cases of IAFI may merely reflect extensive and progressive neural degeneration liberating such amounts as to overcome any presumed threshold created by the blood-brain barrier.

Some investigators have indicated the lack of any abnormal enzyme findings in CNS degenerative disorders(4). No specification of the type of disturbance was noted in these reports. Since an elevated GOT content of CSF seems to be an indication of actively progressive cerebral necrosis rather than a measurement of previously wrought degeneration, it is conceivable that the quiescent phase of a formerly fulminant disease would not show any abnormal GOT findings. The CSF aldolase elevation particularly in the early years of IAFI is probably also explained by continuing destruction of enzyme-bearing neural tissues.

Wróblewski and coworkers have noted that LD activity is increased in the CSF from cases of CNS carcinomatosis, leukemia and lymphoma, but not in patients with primary brain tumor or degenerative CNS disease(10). Application of this procedure to a small number of cases of CNS lipidosis has revealed a significant rise in enzyme activity suggesting that an even wider range of intracellular enzymes are liberated following brain tissue destruction.

Summary. 1) Simultaneous determinations of glutamic oxalacetic transaminase (GOT), aldolase and lactic dehydrogenase (LD) were estimated of sera and cerebrospinal fluid (CSF) derived from 9 cases of infantile Amaurotic Family Idiocy (IAFI), 2 cases of Niemann-Pick's Disease (NPD) and 33 control patients with other neurologic disorders. 2) Parallel values of GOT and LD were invariably elevated in IAFI, while only the serum and CSF GOT were increased in NPD. The CSF aldolase was usually elevated in all cases of CNS lipidosis, while serum aldolase levels were but slightly increased. Significant

CSF enzyme rises were also noted in occasional control patients with actively progressive cerebral degeneration. 3) The data suggest that nervous system necrosis incident to the lipidoses may be biochemically distinguished from other forms of neural degeneration by the analysis of multiple enzyme levels in serum and CSF.

The authors wish to acknowledge the helpful cooperation of Drs. Abram Kanof and Nathan Epstein, for clinical evaluation of patients.

1. Wakim, K. G., Fleisher, G. A., *Proc. Staff Meet., Mayo Clinic*, 1956, v31, 391.

2. Green, J. B., Oldewurtel, H. A., O'Doherty, D. S., Forster, F. M., Sanchez-Longo, L. P., *Neurol.*, 1957, v7, 313.

3. Pearson, C. M., *New Eng. J. Med.*, 1957, v256, 1069.

4. Myerson, R. M., Hurwitz, J. K., Sall, T., *ibid.*, 1957, v257, 273.

5. Aronson, S. M., Saifer, A., Kanof, A., Volk, B. W., *Am. J. Med.*, in press.

6. Karmen, A., Wróblewski, F., LaDue, J., *J. Clin. Invest.*, 1955, v34, 126.

7. Sibley, J. A., Lehninger, A. L., *J. Biol. Chem.*, 1949, v177, 859.

8. Wróblewski, F., LaDue, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 210.

9. Aronson, S. M., Volk, B. W., Epstein, N., *Am. J. Path.*, 1955, v31, 609.

10. Wróblewski, F., Decker, B., Wróblewski, R., *Am. J. Clin. Path.*, 1957, v28, 269.

Received November 22, 1957. P.S.E.B.M., 1958, v97.

Dermal Fibrosis Following Subcutaneous Injections of Serotonin Creatinine Sulphate.* (23734)

RICHARD A. MACDONALD, STANLEY L. ROBBINS, AND G. KENNETH MALLORY
(Introduced by Sidney Farber)
Mallory Institute of Pathology, Boston City Hospital, and Departments of Pathology of Harvard, Tufts, and Boston University Schools of Medicine, Boston, Mass.

5-Hydroxytryptamine (serotonin) has been associated with many physiologic and disease states, but its primary function is at present unknown. One of its most dramatic associations is in patients with the "carcinoid syndrome," in which there are several or all of an unusual group of findings: metastatic carcinoid tumor (argentaffinoma) of the gastrointestinal tract, dyspnea, diarrhea, episodes of flushing and cyanosis, hepatomegaly, ascites and edema, peptic ulcers, and cardiac lesions, chiefly affecting the pulmonic and tricuspid valves. Administration of serotonin to experimental animals has been shown to reproduce the clinical features of the syndrome, and intraperitoneal injections of the serotonin precursor, 5-hydroxytryptophane, have been followed by gastric mucosal erosions(1). It has been hypothesized that the cardiac valvular lesions in the carcinoid syndrome, which consist chiefly of fibrous tissue, are caused by serotonin, and it has been postulated that serotonin elicits a fibrous proliferation of the cardiac valves(2). Up to the present time, however, there has been no experimental evidence to support this hypothesis. In the present study, twice daily subcutaneous injections of serotonin creatinine sulphate resulted in a collagenous and fibrous proliferation in the dermis of rats, with hyperplastic changes of the epidermis. Similar changes were not observed in control animals injected with physiologic saline and with carbon tetrachloride.

Materials and methods. Fifty-three rats of the Sprague-Dawley strain† were injected subcutaneously twice daily for periods of up to 342 consecutive days, using 8 mg doses of

* Aided by grants from Am. Heart Assn. and the Boston Chapter of Mass. Heart Assn.

† Purchased from the Charles River Breeding Laboratories, Cambridge, Mass.

‡ Kindly supplied, in part, by Dr. George Berryman of Abbott Laboratories, North Chicago, Ill., and purchased from the California Fn. for Biochemical Research, Los Angeles.