electrophoretic pattern in the rat and pig further denotes this. The electrophoretic monomorphism exhibited by amylase in guinea pig serum does not necessarily indicate a single derivation site. It may be, rather, that the amylases from the tissues in this animal do not differ sufficiently in charge to make separation possible on paper.

It would be of interest to find, if possible, an explanation for the variable electrophoretic behavior of amylase in the serum of certain mammals. That such differences are not simply the result of binding by serum proteins is suggested by the fact that in some species amylase is demonstrable in electrophoretic areas devoid of stainable quantities of protein. Using a special buffer system, it also has been shown that amylase can be clearly separated electrophoretically from the γ-globulin fraction in human serum(8).

Summary. The electrophoretic distribution of amylase has been examined in sera collected from 11 mammalian species including man. Monomorphic patterns were noted in human, sheep, rabbit, guinea pig, cat and horse serum, whereas amylase behaved polymorphically in specimens from the rat, pig, cow, dog and goat. These findings suggest that, in the latter species, amylase circulates in molecular forms that are distinguishable by electrophoresis on paper. The possible relationships between the paper electrophoretic behavior of serum amylase and tissue derivations are discussed.

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Absence of Serum Alpha-2 Globulin in Niemann-Pick Disease. A Measure of Hepato-Cellular Involvement.* (31386)

LARRY SCHNECK, ABRAHAM SAIFER, HYMAN B. WARSHALL AND BRUNO W. VOLK
Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

Hepatomegaly is one of the major clinical manifestations of Niemann-Pick disease, a sphingomyelin-cholesterol lipidosis(1). Crocker and Farber performed liver function tests on 10 patients with Niemann-Pick disease. They found a poor correlation between involvement of the organ, and the various flocculation tests, bromsulfalein excretion, and serum protein concentration and distribution (2). They concluded that "there is considerable need for laboratory procedures that would give a more profound evaluation of the undoubted hepatic malfunction present in this disease." Although serum alpha-2 globulin is frequently decreased in primary liver disease when measured by paper electrophoresis with a veronal buffer(3), Crocker and Farber found that the electrophoretic pattern in Niemann-Pick disease was normal. Since Tris borate buffer fractionates the alpha globulins into 3 distinct bands, compared to only 2 obtained with veronal buffer, the sera of 10 patients with Niemann-Pick disease were analyzed by paper electrophoresis with the Tris borate buffer. It was observed that alpha-2 globulin (the second of the 3 alpha bands) was absent in all 10 patients. A similar pattern was obtained from 13 out of 14 sera of patients with primary or secondary

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liver disease, and from the sera of 3 out of 8 parents of children with Niemann-Pick disease. This fraction was absent in several patients with hepatic disease even though the serum glutamic pyruvic and oxaloacetic transaminase activities were normal.

Patient material. The study was carried out on 10 patients with Niemann-Pick disease (varying in age from 5 months to 10 years), as well as on a control group of 60 individuals, 20 of whom were children under the age of 5 years. All 10 patients, diagnosed as Niemann-Pick disease, had characteristic Niemann-Pick cells in aspirated bone marrow. Eight of the 10 patients were of Jewish origin. The non-Jewish patients, sister and brother, were 7 and 10 years old respectively. In 9 of the 10 cases, the disease was clinically apparent by the end of the first year. One patient, the 7-year-old non-Jewish girl, was diagnosed at age 5. This child showed mild mental retardation but has no evidence of hepatosplenomegaly.

The control group consisted of 60 adults and children. This group was comprised of 14 patients with primary or secondary liver disease; 8 parents of Niemann-Pick patients; 12 children (age 8 months to 4 years) with neurological disease; as well as 6 hospitalized children (under the age of 3) and 20 hospitalized adults (ages 20 to 70) with no clinical signs of neurologic or hepatic disease (Table I).

TABLE I. Serum Protein Pattern of 10 Niemann-Pick Patients and 60 Controls (Paper Electrophoresis with Tris Buffer).

Diagnosis	No. of patients	No. of patients with absent serum alpha-2 globulin
1. Niemann-Pick disease	10	10
2. Liver involvement		
a. Laennec's cirrhosis	5	5
b. Hepatitis	4	4
c. Carcinoma, primary or secondary	4	3
d. Infantile Gaucher's disease	1	1
3. Parents of Niemann-Pick disease patients	8	3
4. Neurological diseases		
a. Tay-Sachs disease	7	0
b. Late infantile lipidosis	1	0
c. Spongy degeneration of central nervous system	1	1
d. Cerebral palsy	3	0
5. Miscellaneous hospitalized children		
a. Acute respiratory disease	5	0
b. Lymphocytic leukemia	1	0
6. Miscellaneous hospitalized patients	20	2*

* Macrocytic anemia.

Paper electrophoresis method. Ten lambda of fasting serum were analyzed by paper electrophoresis using a Tris buffer.† The paper strips were run horizontally in tanks containing the Tris buffer, at 185 volts, for 20 hours. The proteins ran from the negative to positive electrode. The strips were removed and dried in an oven for 30 minutes at 100°C. The protein stain used was Lissamine green (0.3% Lissamine green in 15% acetic acid). The paper strips were stained for 10 minutes, the excess stain poured off and the strips then washed with 2% acetic acid until the background was white. They were then placed on filter paper to dry. The serum glutamic pyruvic transaminase and the serum glutamic oxaloacetic transaminase were determined by the methods of Karmen, Wroblewski and La Due(4), and Mohun and Cook(5).

Results. The normal serum protein pattern, obtained by paper electrophoresis with Tris borate buffer, consisted of albumin, and alpha-1, alpha-2, alpha-3, beta-1, beta-2, and gamma globulin (Fig. 1). In all 10 patients with Niemann-Pick disease, the alpha-2 band was absent (Fig. 1). This fraction was also absent in 13 out of 14 patients with primary or secondary liver disease; in 3 out of 8 parents of Niemann-Pick children; in a child with spongy degeneration of the white matter; and in 2 adults with macrocytic anemia.

One of the Niemann-Pick patients with absent alpha-2 globulin had a normal serum glutamic oxaloacetic transaminase and another one had a normal serum glutamic pyruvic transaminase level. The alpha-2 globulin was also absent in 3 patients with neo-

† 484 g of Sigma 7-9 (Tris), 48 g of EDTA, 36 g of boric acid, diluted to 2 liters with distilled water. 750 cc of the stock solution were then diluted to 2 liters with distilled water.

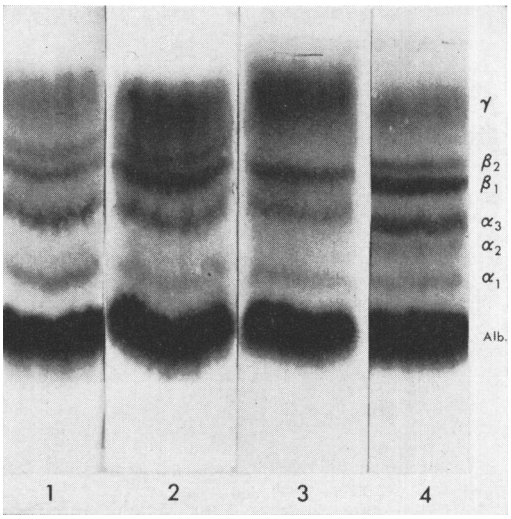

FIG. 1. Serum electrophoretic pattern. 1. 9-year-old boy with Niemann-Pick disease and hepatomegaly. 2. 7-year-old girl with Niemann-Pick disease and no hepatomegaly. 3. Mother of a child with Niemann-Pick disease. 4. 3-year-old boy with Tay-Sachs disease.

plastic involvement of the liver. One of these 3 had normal serum glutamic pyruvic and oxaloacetic transaminase levels, and another had normal serum glutamic oxaloacetic transaminase but elevated glutamic pyruvic transaminase activity. The serum glutamic pyruvic and oxaloacetic transaminase activities were normal in the child with infantile Gaucher's disease (Table II).

Discussion. The present study shows absence of the serum alpha-2 globulin fraction in all 10 cases with Niemann-Pick disease. This is in contrast to the observations of Crocker and Farber who reported a normal electrophoretic serum pattern in a case afflicted with this disorder(2). Since their study was published in 1958, it is assumed that they did not use a Tris buffer. This buffer fractionates the alpha-2 globulins in 3 distinct bands, compared to the 2 bands obtained with the commonly used veronal buffer.

The absence of serum alpha-2 globulin is characteristic, but not diagnostic of Niemann-Pick disease. A similar protein pattern was observed in 3 of the 8 parents of children with Niemann-Pick disease, in 3 hospitalized patients with no clinical evidence of liver disease, and in 13 out of 14 cases with primary or secondary liver involvement. The latter findings are in keeping with the observation that the serum alpha-2 proteins are decreased in liver disease when analyzed by paper electrophoresis with veronal buffer (3).

On the basis of tests performed to detect liver involvement in Niemann-Pick disease, the absence of serum alpha-2 globulin on paper electrophoresis with Tris buffer appears to be a most sensitive indicator of the hepatic involvement. Serum flocculation, total bilirubin, total cholesterol, and total albumin and globulin were measured in 5 Niemann-Pick patients. Except for an elevated thymol turbidity in one patient, the other tests were all within normal limits. These results are similar to those obtained by Crocker and Farber, who also reported normal bromsulfalein ex-

TABLE II. Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase in Niemann-Pick Disease and in Patients with Primary and Secondary Liver Involvement.

	Serum glutamic oxaloacetic transaminase (normal 10-40 u)	Serum glutamic pyruvic transaminase (normal 5-35 u)
Niemann-Pick disease		
Hepatomegaly (5 mo)	220	560
" (12 ")	226	552
" (14 ")	292	620
" (20 ")	500	550
" (22 ")	333	470
" (5 years)	3.6	50
No hepatomegaly (7 ")	5.4	30
Hepatomegaly (9 ")	132	40
Liver disease		
A. Laennec's cirrhosis		
Patient 1	30	18
" 2	58	31
" 3	117	18
B. Viral hepatitis (acute)		
Patient 1	337	708
" 2	178	664
" 3	351	38
C. Carcinoma		
1. of gall bladder with extension to liver	48	43
2. of colon with liver metastases	23	27
3. Metastatic carcinoma of liver with unknown primary tumor	31	89
D. Infantile Gaucher's disease	40	12

cretion in a child with Niemann-Pick disease(2). Furthermore, the alpha-2 globulin was absent in children with Niemann-Pick disease and in patients with primary and secondary liver involvement even when the serum glutamic oxaloacetic and pyruvic transaminase were within normal limits (Table II).

There is no ready explanation for the absence of serum alpha-2 globulin in 3 hospitalized patients with no clinical signs of liver disturbance, and in 3 out of 8 apparently healthy parents of Niemann-Pick children. If a small percentage of the general population normally has absent serum alpha-2 globulin (as measured by paper electrophoresis with Tris buffer) then the pattern obtained in these 6 subjects could have occurred by chance. However, the fact that this fraction was absent in 3 out of the 8 parents suggests that the heterozygote state of Niemann-Pick disease may be associated with some variation of protein synthesis.

Summary. The sera of 10 children with Niemann-Pick disease, 9 of whom had hepatomegaly, were analyzed by paper electrophoresis with a Tris borate buffer. The alpha-2 globulin fraction was absent in all 10 sera. This alpha globulin fraction was also absent in 13 out of 14 control patients with primary or secondary liver involvement. The absence of serum alpha-2 globulin appears to be a sensitive indicator of liver involvement, since it is absent even when serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase are normal. Six of the remaining 46 controls with no evidence of clinical liver disease also had absent serum alpha-2 globulin. Three of these were parents of the children with Niemann-Pick disease.

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