2. Burke, J. D., *Physiol. Zool.*, 1954, v27, 1.
3. Friedman, J. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 323.
4. Leonard, S. L., *ibid.*, 1957, v96, 720.
5. Berg, L., Ebaugh, Jr., F. G., Shy, G. M., Horvath, B., Cummings, D. J., *J. Appl. Physiol.*, 1955, v8, 31.

Received October 20, 1958. P.S.E.B.M., 1959, v100.

Localization of 5-Nucleotidase and Non-specific Alkaline Phosphatase By Starch Gel Electrophoresis. (24568)

O. DHODANAND KOWLESSAR, JAMES H. PERT,* LORRAINE J. HAEFFNER AND MARVIN H. SLEISENGER (Introduced by A. Mazur)
Department of Medicine, N. Y. Hospital-Cornell Medical Center, N. Y. City

Bone, intestine, and possibly liver produce non-specific alkaline phosphatase. A specific alkaline phosphatase, 5-nucleotidase, is present in normal serum(1). It is elevated in sera of patients with biliary cirrhosis and obstructive jaundice of extra hepatic origin, and normal in those with bone diseases(1). The present work describes the electrophoretic separation of serum 5-nucleotidase and non-specific alkaline phosphatase by the starch gel technic. These experiments are part of an integrated study of the biochemical and kinetic properties of these enzymes.

Methods. All sera were obtained in the fasting state. The study group included 6 normal adults and 3 normal children, and 20 patients with a variety of disease states, particularly those associated with elevation of serum non-specific alkaline phosphatase. In the latter group were 5 patients with Paget's disease, 2 with osteomalacia secondary to malabsorption, 5 with biliary cirrhosis, 3 with Laennec's cirrhosis, 3 with biliary obstruction secondary to choledocholithiasis and carcinoma, and 2 with sarcoidosis. Non-specific alkaline phosphatase and 5-nucleotidase were determined in sera and starch gel by the method of Dixon and Purdom(1) with the following modifications: 0.3 ml of 0.05 M $MgSO_4$ was substituted for 0.3 ml of water in the pH 9.3 and 7.5 β-glycerophosphate substrate systems, while 0.3 ml of 0.4 M $MgSO_4$ was used for the pH 7.5 adenosine-5-phosphate substrate system. Serum proteins were separated by zone electrophoresis according to a modification of the method of Smithies(2,3). Idaho potato starch† was hydrolyzed for 70 minutes at 36.4°C and dried to a moisture content of 10.3%. The gel was prepared by heating 13.5 g of this soluble starch with 100 cc of 0.027 M borate buffer, pH 9.05. Electrophoresis was carried out at 12°C at 4.5 volts per cm for 16 hours. The gels were then scored in ½ cm sections and cut in half along the horizontal plane. One half was retained for protein staining while the second half was sectioned in ½ cm blocks and placed directly into tubes containing the respective buffer, substrate, and required Mg concentrations.

Results. In sera of both normal subjects and of patients with eleavted non-specific alkaline phosphatase, there appear to be 2 alkaline phosphatases. One of these migrates in the region of alpha-2-globulin and beta-lipoprotein, the other with the beta-globulin components of serum (Fig. 1). Approximately 60% of the alkaline phosphatase migrating with the alpha-2-globulin and beta-lipoprotein is made up of 5-nucleotidase. There is no 5-nucleotidase activity in the alkaline phosphatase that migrates with the beta-globulin fraction (Fig. 1). Total non-specific alkaline phosphatase and 5-nucleotidase recoveries in these starch gel blocks were 90% and 40% of the serum used, respectively. Since borate is a known inhibitor of non-specific alkaline phosphatase(4), we attempted to show the relationship of borate inhibition and its correction by addition of Mg ions. It can be seen from Table I that the inhibition of alka-

* Present address: Connaught Laboratories for Medical Research, Univ. of Toronto, Canada.

† Batch #584, Magic Valley Processing Co., Twin Falls, Idaho.

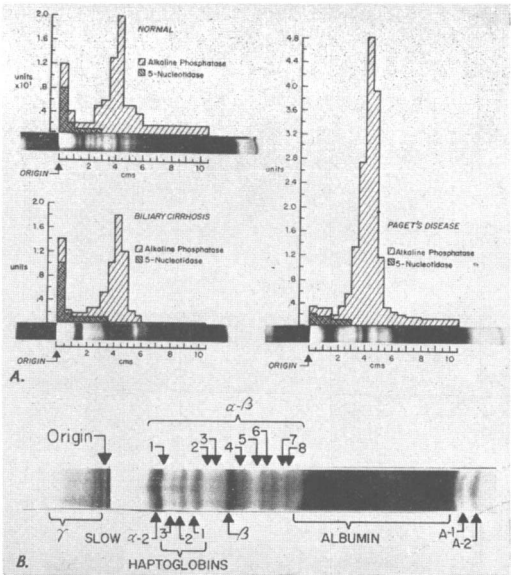

FIG. 1. Localization of 5-nucleotidase and non-specific alkaline phosphatase by starch gel electrophoresis. A. Demonstrating sites for these enzymes in normal individual and in patients with biliary cirrhosis and Paget's disease. B. Starch gel electrophoregram of normal serum for reference.

line-phosphatase is corrected by the Mg concentration used, while there is still 50% inhibition of the 5-nucleotidase even with 0.4 M Mg used in our test system. We feel that this accounts for the 40% yield of 5-nucleotidase found utilizing starch gel electrophoretic separations with 0.027 M borate buffer.

Discussion. Results of paper electrophoresis indicate that the locus of non-specific alkaline phosphatase coincides exactly with that of alpha-2-globulin(5,6). Recently, Fahey *et al.* have separated 2 alkaline phosphatases by an anion-exchange cellulose chromatographic method(7). Our results clearly demonstrate 2 electrophoretically separable alkaline phosphatases in both normal sera and sera of pathological states. One is found in the region of beta-lipoprotein and alpha-2-globulin, the other with beta-globulin. The alkaline phosphatase that coincided with the latter fraction of serum occurred in all cases studied and was increased strikingly in patients with Paget's disease and biliary cirrhosis. A similar but less exaggerated peak is seen in healthy children and in patients with Laennec's cirrhosis, obstructive jaundice due to stone and carcinoma, osteomalacia secondary to malabsorption, lymphoma, and sarcoidosis. Since the electrophoretic mobilities are consistently similar in all cases studied, and since there is no 5-nucleotidase present in this beta-globulin peak, it would appear that this enzyme represents alkaline phosphatase derived from bone. The remaining alkaline phosphatase migrates with the alpha-2-globulin and beta-lipoprotein and is made up of both 5-nucleotidase and non-specific alkaline phosphatase. Since there is no 5-nucleotidase in bone, and since level of serum 5-nucleotidase was normal in diseases of the bone, it would appear that the source of this enzyme is probably the liver.

Summary. 1. By means of starch gel electrophoresis, 2 alkaline phosphatases were demonstrated, one migrating in the region of alpha-globulin and β-lipoprotein and the other with beta-globulin. 2. 5-nucleotidase consti-

TABLE I. Effect of Magnesium Ions on Inhibition of Serum 5-Nucleotidase and Non-Specific Alkaline Phosphatase by Borate.

Enzymes		Conc. borate, M	Conc. Mg, M	Units of enzyme activity	% inhibition of control
Alk. p'tase,	pH 9.3	0	5×10^{-2}	4.3	0
5-Nucl.	7.5	0	4×10^{-1}	1.64	0
Alk. p'tase	9.3	$.625 \times 10^{-2}$	5×10^{-2}	4.3	0
5-Nucl.	7.5	"	4×10^{-1}	1.2	24
Alk. p'tase	9.3	1.25×10^{-2}	5×10^{-2}	4.3	0
5-Nucl.	7.5	"	4×10^{-1}	1.2	24
Alk. p'tase	9.3	2.5×10^{-2}	5×10^{-2}	4.2	2
5-Nucl.	7.5	"	4×10^{-1}	.8	50
Alk. p'tase	9.3	2.5×10^{-2}	0	3.4	21
5-Nucl.	7.5	"	0	.5	70

Alk. p'tase = Alkaline phosphatase. 5-Nucl. = 5-Nucleotidase.

tuted 60% of the former, but was not present in the latter. 3. Although alkaline phosphatase which migrated with beta-globulin was elevated in both diseases of bone and liver, it is thought to be derived from bone since it had no 5-nucleotidase. 4. Since 5-nucleotidase is not contained in bone and is not elevated in diseases of the bone, it is thought to be derived from the liver.

1. Dixon, T. F., Purdom, M., *J. Clin. Path.*, 1954, v7, 341.

2. Smithies, O., *Biochem. J.*, 1955, v61, 629.

3. Pert, J. H., Sleisenger, M. H., Woods, K. R., Engle, R. L., Jr., *Clin. Res. Proc.*, 1957, v2, 156.

4. Colowick, S. P., Kaplan, N. O., *Methods in Enzymology*, 1955, v2, 1955.

5. Baker, R. W. R., Pellegrino, C., *Scand. J. Clin. and Lab. Invest.*, 1954, v6, 94.

6. Wolfson, W. Q., *Nature*, 1957, v180, 550.

7. Fahey, J. L., McCoy, P. F., Goulian, M., *J. Clin. Invest.*, 1958, v37, 272.

Received November 5, 1958. P.S.E.B.M., 1959, v100.

Effect of Neomycin on Serum Cholesterol Level of Man.* (24569)

PAUL SAMUEL‡ AND ALFRED STEINER (Introduced by David Seegal)

First (Columbia University) Division, Goldwater Memorial Hospital, Dept. of Hospitals and Dept. of Medicine, College of Physicians and Surgeons, Columbia University, N. Y.

During a clinical study of the effect of various dietary fats on the serum lipids of a group of patients with coronary atherosclerosis, it was observed that oral administration of neomycin to a patient with a gastrointestinal infection due to Salmonella Tennessee was associated with a significant fall in serum cholesterol concentration. The decrease in serum cholesterol level occurred at a time when the patient had clinically recovered from the infection although the organism was still present in the stools. To evaluate the significance of this observation, the effect of neomycin administered orally on the serum lipids was studied in 10 additional patients, none of whom were known to have disease of the gastro-intestinal system.

Method. The study group consisted of 6 male and 4 female patients ranging in age from 21 to 72 years. Nine were hospitalized and one was observed as an outpatient. Clinical diagnosis of each subject is listed in the Table. Total serum cholesterol levels were determined twice weekly in each patient by the method of Abell *et al.*(1). The ester fraction was determined by the method of Schoenheimer and Sperry(2); and total serum lipids by a gravimetric procedure(3). Five of the hospitalized individuals were on the regular hospital diet in which 45% of the calories are derived from fat. Of the remaining 5 patients, one (Nas) received a diabetic diet of C 180, P 80, F 80 g without insulin; one (Ber), with familial hypercholesterolemia, was on a low fat diet in which 25% of the calories were derived from fat; 2 patients were maintained on a similar low fat diet after a preliminary period of 6 weeks on the regular hospital diet; and the outpatient ate her usual diet without restriction during the study. Average pretreatment serum lipid values were established during periods of 5 weeks or longer prior to oral administration of neomycin. Neomycin was given as Mycifradin Sulfate®.† This preparation contains 70% neomycin sulfate. The doses of medication in this study represent the weight of Mycifradin Sulfate. Six patients were given 2 g each day. The remaining 4 patients received from 0.5 to 2 g daily. The period of neomycin administration varied from 3 to 16 weeks. If the amount of drug was varied, it was usually done so after 4 week periods at each dose level.

Results. The results of oral administration of neomycin on the serum cholesterol level are

* Aided in part by grants from N. Y. Heart Assn., Albert and Mary Lasker Fn., Thomas Phillips Fund and Revlon Fund.

‡ Present address: Dept. of Med., New York Univ. Med. Center.

† The authors are indebted to Upjohn Co. for generous supply of Mycifradin Sulfate.