Localization of Serum Leucine Aminopeptidase Activity by Paper Electrophoresis* (27617)

EDGAR E. SMITH, ESTEBAN P. PINEDA, AND ALEXANDER M. RUTENBURG
(Introduced by Jacob Fine)
Surgical Research Laboratory, Yamins Research Laboratory, Beth Israel Hospital, and Departments of Surgery, Beth Israel Hospital and Harvard Medical School, Boston

There is evidence(1-3) that a given enzymatic activity of serum may represent a summation of the activities of several discrete components. The individual components having in common the ability to catalyze a particular biochemical reaction may differ from one another in other respects. Thus, components may exhibit the same substrate specificity while differing in certain other properties, *e.g.*, rate of reaction with certain cofactors, heat stability, or electrophoretic mobility. The term "isoenzymes" as currently employed generally refers to components resolved by electrophoresis(2). A significant outgrowth of this demonstration of isoenzymes has been the implication that a study of these components in serum and tissues may complement the present methods of total enzyme assay in diagnosis and localization of diseased tissues. The reported electrophoretic resolutions of lactic dehydrogenase and leucine aminopeptidase† (LAP) utilized the starch gel technic. This is a method which is not easily adaptable to routine use in a clinical laboratory. The present investigation was, therefore, undertaken to study the usefulness of paper electrophoresis for demonstration of different forms of LAP in human serum with a view to providing a more facile technic for identification of isoenzymes.

Methods. Serum samples were divided into 2 portions. One was assayed routinely for total serum LAP activity by a method described elsewhere(4). The second was electrophoresed on Whatman #3 MM paper strips, 6 cm × 30.6 cm. Each strip was divided horizontally into two 3-cm portions by a pencil line drawn through the center of the paper and vertically by a line drawn through the strip 15.3 cm from the edge. Each strip was then divided by vertical parallel lines into 25 numbered rectangular sections (0.5 cm × 6 cm), 19 on the anodal side of the vertical center and 6 on the cathodal side. The strips were placed in a Durrum type electrophoresis cell‡ and 10 μl of serum were placed on the center line (between sections 19 and 20) of each 3-cm half of each strip. Electrophoresis was carried out overnight (16-17 hours) in Spinco B-2 Veronal buffer, pH 8.6, ionic strength 0.075 at a constant current of 6 m. amps. for 4 strips. Following electrophoresis the strips were divided horizontally into two 3-cm segments. One segment was stained for protein by the bromphenol blue method(5).

The remaining segment was cut into twenty-five 0.5-cm sections (as marked). Each of the smaller sections was then cut into 5-6 smaller pieces and placed in a small test tube containing 1 ml of LAP substrate (L-leucyl-β-naphthylamide) solution and assayed for activity as described elsewhere(4) with the exception that after addition of trichloroacetic acid all other reagents were added in one-half the usual amounts. LAP activity was localized in the various serum protein fractions by matching the 0.5-cm sections showing LAP activity with appropriate counterparts on the strip stained for protein.

Human bile was electrophoresed in a similar manner.

Results. The electrophoretic localization of LAP in serum from normal individuals and in patients with normal serum levels differed from that in patients with hepatobiliary or pancreatic disease and elevated serum LAP activity (Table I). Thus, in normal serum,

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† As determined by hydrolysis of L-leucyl-β-naphthylamide. Results obtained with this substrate and their interpretation differ from those obtained with L-leucylglycine.

‡ Spinco.

TABLE I. Localization of LAP in Serum Fractions Following Electrophoresis on Paper. Figures in parentheses represent numbers of patients having activity in the designated fraction. Plus sign alone indicates activity in all patients.

Diagnosis	No. studied	Serum LAP range (units)	Distribution of serum LAP				
			Albumin	α_1	α_2	β	Origin
Normal	10	127- 183		+			
Ca head pancreas	18	279-1296		+		+ (14)	+ (2)
Choledocholithiasis	18	333-1400	+ (13)	+		+ (4)	
Acute hepatitis	6	249-1624	+ (1)	+	+ (2)	+ (2)	
Drug-induced jaundice	5	379-1222		+		+ (4)	+ (1)
Liver metastases							
a. Icteric	10	226- 410	+ (2)	+	+ (2)	+ (8)	+ (7)
b. Anicteric	8	460-2240	+ (2)	+	+ (1)	+ (4)	+ (1)
Pancreatitis	4	136- 465		+			
Cirrhosis							
a. Icteric	11	311-1712		+	+ (6)	+ (9)	
b. Anicteric	5	119- 365		+			
Pregnancy							
a. 1st trimester	3	109- 163		+			
b. 3rd "	3	488- 868			+		
Biliary atresia	6	590-1002		+		+	+ (2)

LAP migrated as a single component with the mobility of the α_1-globulin (Fig. 1). Patients with diseases of the liver, biliary tract or pancreas also showed activity in the α_1-globulin region but additional peaks of LAP activity were localized in other serum fractions.

Since LAP activity was observed throughout the electrophoregrams of most abnormal sera, a value of 40 Klett units was found most suitable for a baseline activity and increments therefrom in the various protein fractions were taken as abnormal elevations. The activity observed in the α_1-globulin was observed as a distinct peak. Activity in the albumin fraction appeared to represent an overlap of the α_1-globulin activity since only one peak was observed in the region of these two fractions. Activity in the α_2-globulin appeared either as a distinct peak or a spread to the β-globulin. Activity in the β-globulin also appeared as a distinct peak or spread to the origin.

The activity at the origin was not a definite peak but rather a spread from the β- or α_2-globulin to the origin. Such a spread may result from irreversible adsorption of the serum enzyme on the paper during electrophoresis.

Extrahepatic obstructive jaundice. The pattern of serum LAP activity in obstructive jaundice varied with the nature of the obstruction. In most patients with cancer of the head of the pancreas preoperatively, additional LAP activity was observed as a peak in the β-globulin region (82%), accompanied in a few cases by activity at the origin (11%). None showed activity in the albumin or α_2-globulin fractions. Following surgical relief of biliary obstruction the decrease in serum LAP activity was paralleled by the disappearance of the β-globulin peak (Fig. 2). In choledocholithiasis the typical pattern was one of activity in the α_1-globulin and in the albumin fractions. There was no activity in the α_2-globulin or origin (Fig. 3).

Hepatocellular jaundice. Sera of icteric patients with acute hepatitis and drug-induced jaundice exhibited additional LAP activities either in the α_2- or β-globulin, albumin or origin. In patients with hepatocellular jaundice the appearance of LAP activity only in the α_1-globulin region coincided with the decline in total serum LAP activity and clinical improvement.

Eight of 10 patients with hepatoma or hepatic tumor metastases showed an additional peak in the β-globulin (Fig. 4). Five of these patients also showed activity at the origin, and 2 exhibited activity in the albumin fraction and at the origin. The remaining

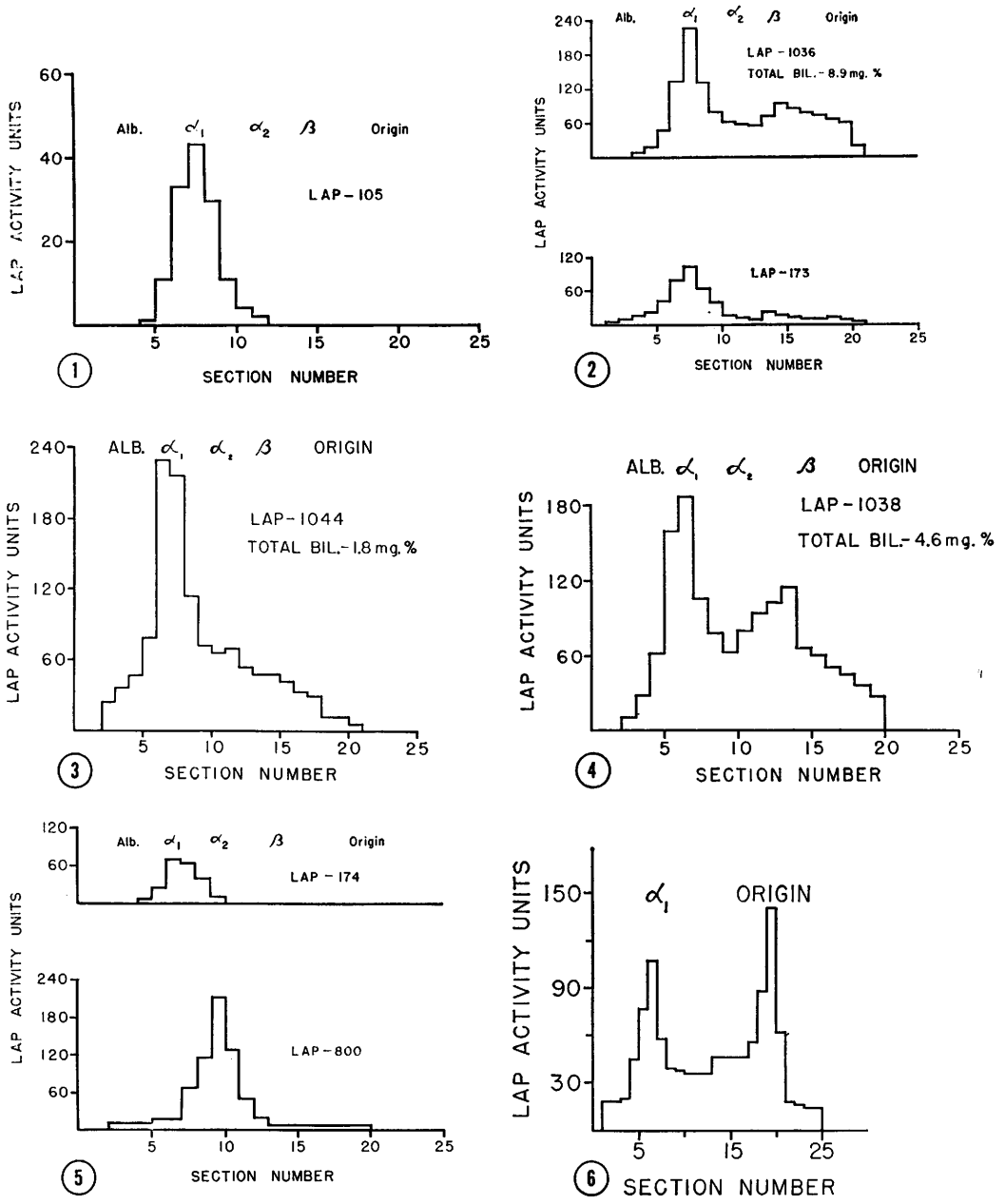

FIG. 1. Electrophoretic pattern of LAP in normal serum.

FIG. 2. Electrophoretic pattern of LAP in serum from patient with common duct obstruction due to carcinoma of head of pancreas. Top: Before operation. Bottom: Following surgical removal of obstruction.

FIG. 3. Electrophoretic pattern of LAP in serum from patient with choledocholithiasis.

FIG. 4. Electrophoretic pattern of serum LAP from a patient with cancer of prostate with liver metastases.

FIG. 5. Electrophoretic pattern of serum LAP in pregnancy. Top: First trimester. Bottom: Third trimester.

FIG. 6. Electrophoretic pattern of LAP in human bile.

2 patients showed additional activity only in the α_2 region.

Anicteric patients. The electrophoregrams in chronic pancreatitis with normal serum levels were similar to those in normal subjects, *i.e.*, with LAP activity only in the α_1-globulin. Three of 8 anicteric patients with hepatic tumor metastases showed activity only in the α_1-globulin, but 4 others exhibited additional activity in the β-globulin (2 of which showed activity in the albumin) and the 8th had activity both in the α_2 and origin. One of 4 patients with congestive heart failure showed additional activity in the albumin region.

Cirrhosis. The electrophoretic pattern of serum LAP in cirrhosis appeared related to the presence or absence of jaundice. Anicteric patients with cirrhosis exhibited LAP activity only in the α_1-globulin fraction. However, in jaundiced patients additional activity was observed in the α_2- and/or β-globulin regions.

Pregnancy. In the first and second trimester of pregnancy serum electrophoresis showed activity in the α_1-globulin, but in the third trimester the activity shifted from the α_1-globulin to the α_2-globulin (Fig. 5). This change in electrophoretic mobility closely paralleled the increase in total serum LAP activity.

Bile. The electrophoretic localizations of LAP in bile specimens (obtained at operation or from T-tube drainage) in 6 patients all showed essentially the same pattern, namely a peak in the α_1-globulin region accompanied by a definite peak at the origin (Fig. 6). Due to the very low concentration of protein in bile, the peak at the origin may result from adsorption of enzyme at this site. The peak observed in the region of the α_1-globulin corresponded with that seen in the serum.

Discussion. Using paper electrophoresis the results herein reported indicate that LAP in normal serum migrated as a *single* component with the mobility of an α_1-globulin. Augmentation of the α_1-globulin activity, together with frequent appearance of additional LAP components or "isoenzymes" in other serum fractions, was observed in the sera of patients with increased serum LAP levels due to diseases of the liver, biliary tract, or pancreas. The isoenzymes demonstrable in the sera of patients with liver disease may be due to biliary obstruction or abnormal synthesis of the enzyme in liver.

Preliminary studies with tissue LAP indicate that the electrophoretic mobilities of LAP in liver and serum were identical.§ However, additional isoenzymes were also demonstrable in other extrahepatic tissues, such as pancreas, kidney, and placenta. Their contribution to serum LAP activity in health and disease is currently under investigation.

The paper electrophoresis patterns of serum LAP are of limited diagnostic usefulness and in jaundiced sera were largely related to the presence or absence of activity in the albumin, β-globulin or at the origin. More constant patterns were encountered in pregnancy, common duct stone, and cancer of the head of the pancreas. The LAP activity in regions other than the α_1-globulin found in the sera of patients with common duct obstruction and liver disease disappeared with relief of obstruction or with biochemical and clinical improvement of liver disease. The demonstration and identification of the different forms of LAP in serum require electrophoretic fractionation and immunoelectrophoretic studies of tissue LAP to localize specifically the source or sources of these "isoenzymes". This investigation is currently in progress.

Summary. The electrophoretic behavior of serum leucine aminopeptidase (LAP) in normal individuals, in pregnancy, and in patients with hepatobiliary disease was investigated with paper electrophoresis technics. In normal serum LAP migrated as a single component in the α_1-globulin fraction. In patients with elevated serum LAP activity due to hepatobiliary or pancreatic disease supplementary peaks of enzymatic activity were demonstrable in other protein fractions. Thus, in jaundiced patients with carcinoma of the head of the pancreas, liver metastases, biliary atresia, acute hepatitis, and drug-induced jaundice, additional activity was observed in the β-globulin fraction. In some patients with hepatitis additional activity was observed in the α_2-globulin as well as β-globu-

§ To be published.

lin. In common duct stone additional activity in the albumin fraction was a consistent finding. In early pregnancy the electrophoretic pattern of LAP was similar to that observed in normal serum, whereas during the third trimester LAP activity was demonstrable only in the α_2-globulin.

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Further Observations on Induction of Tumors in Mice with Radioactive Thymidine.* (27618)

RENATO BASERGA, HERMANN LISCO AND WALTER KISIELESKI
(Introduced by F. Wassermann)
Department of Pathology, Northwestern University Medical School, Chicago and Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill.

We have reported(1) preliminary results of an experiment on induction of tumors in mice with radioactive thymidine. It was shown that tumors could be induced in mice with a single injection of tritiated thymidine at a dose level of 1.0 μc/g of body weight. These studies have been continued and the earlier results confirmed and extended. We wish to summarize here all of the presently available data, including observations on mice injected with thymidine labeled with carbon-14 and other data not mentioned earlier.

Four aspects of tumor induction and life shortening by radioactive thymidine were studied as follows (Table I): 1. Effect of tritium labeled thymidine as a function of age of the animal at the time of injection (Groups 1-3); 2. Effect of tritium labeled thymidine as a function of dose and dose-fractionation (Groups 5-8); 3. Effect of carbon-14 labeled thymidine as a function of dose and dose-fractionation (Groups 11-14); 4. Effect in offspring of mice injected with tritium labeled thymidine during pregnancy (Group 10).

Methods. F_1 hybrids of C and A mice, whose lines have been maintained at Northwestern University for more than 5 years, were housed in plastic cages, in air-conditioned quarters, and fed Rockland Mouse Diet and water. The H^3-thymidine (New England Nuclear Co., Boston, Mass.) had a specific activity of 360 mc/mM, while the thymidine-2-C^{14} (New England Nuclear Co.) had a specific activity of 3.0 mc/mM. Non-radioactive thymidine, M.W. 242.2, was obtained from the California Corp. for Biochemical Research, Los Angeles. The required amount of thymidine injected was dissolved in 0.1 ml of sterile water per mouse. Adult mice were injected intraperitoneally, and newborn mice subcutaneously in the suboccipital region, at the dose-levels of thymidine indicated in Table I. An additional group of mice consisted of the offspring of pregnant mice injected with 250 μc of H^3-thymidine between the 15th and 17th day of pregnancy. All animals received a single injection of radioactive thymidine, with the exception of Groups 8 and 14, which received thymidine in 6 fractionated doses spread over a period of 8 days. Control mice were in-

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