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Separation of Leucylaminopeptidase Components in Normal Human Sera by DEAE Cellulose Column Chromatography. (27221)

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As a part of a general program to study the significance of differences of various enzyme activities in serum, a systematic study of leucylaminopeptidase (LAP) in human tissues has been initiated. In the literature there are conflicting opinions about the meaning of altered serum leucylaminopeptidase levels (SLAP). Rutenburg, Pineda, *et al.*(1,2) have proposed that elevated SLAP levels have considerable significance in the diagnosis of carcinoma of the head of the pancreas, whereas Shay *et al.* and Winsten(3,4) maintain that elevated SLAP levels are of little value in this connection. Kowlessar *et al.*(5) and Rutenburg *et al.*(6) have reported the appearance of second and third SLAP components in addition to the "normal component" in patients with hepatobiliary disease. This report will deal specifically with our studies of these SLAP components.

Materials and methods. LAP activity was determined by a modification of the method of Goldbarg(7). The unit of LAP activity is defined as that amount of enzyme that will liberate one microgram of beta naphthylamine from the substrate, L-leucyl-beta-naphthamide, in one hour at 37°C.

Serum samples were fractionated by means of DEAE Cellulose (DEAE Cel) chromatography. Prior to application to the column the samples, 2.0 ml of serum, were dialyzed at 4°C against 100 volumes of 0.005 M phosphate buffer at pH 8.6. DEAE Cel, Type 40, was obtained from Carl Schleicher & Schuell Co. This adsorbant was equilibrated with the above buffer before use. The columns used were 11 mm in diameter and 40 cm long. The column was then eluted at 4°C by means of a gradient elution device that provided a decrease in pH from 8.6 to 4.0 of a 0.005 M phosphate buffer and an increase in sodium chloride concentration from zero to 0.18 M in the course of the delivery of 400 ml of phosphate-sodium chloride solution to the column. Fraction volume was 6.0 ml.

Results. In our laboratories, we have subjected the sera of 22 normal adult persons to DEAE Cel chromatography. It has been found that in the serum of all such individuals, there were 4 distinct SLAP components. In Fig. 1, the elution patterns for a representative normal adult subject and a person with severe obstructive jaundice are presented. The components have been arbitrarily identified in the order of elution, and are defined as follows: α_1 LAP, activity in fractions 25-39; β_1 LAP, activity in fractions 40-50; β_2 LAP, activity in fractions 51-58; and β_3 LAP, activity in fractions 59-74.

There is some variation in amount of this enzyme activity in each of the SLAP components in sera of normal subjects. The most constant component is the first one to be eluted, namely, α_1-LAP, which has always been found to account for a large proportion of total SLAP activity. In Table I, mean and extreme concentrations for each of the SLAP components in normal sera are listed.

The elution pattern (Fig. 1) of a person with obstructive jaundice is typical of 7 cases of elevated SLAP screened so far. These include carcinoma of the pancreas, cholelithia-

sis, and carcinoma of the pancreas with metastasis to the liver. The elevation in SLAP appears to be an increase in all LAP components, rather than the appearance, *de novo*, of other components in cases of hepatobiliary disease.

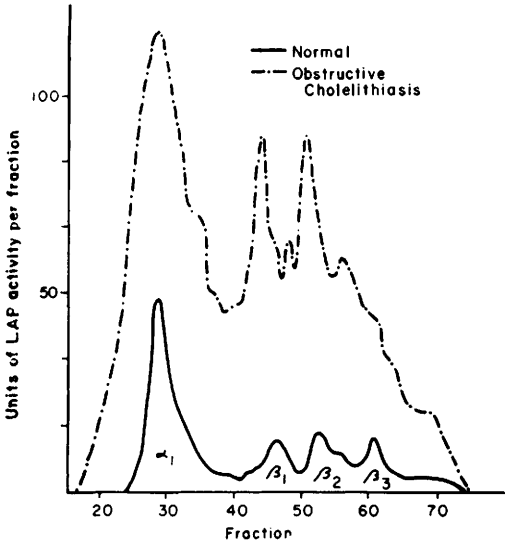

FIG. 1. LAP activity in fractions of typical serum samples from (1) a normal person and (2) a person with obstructive cholelithiasis.

TABLE I. Average and Range of Concentrations of SLAP Components in Serum of Normal Persons.*

Component	Avg level (units/ml)	Extreme levels (units/ml)
α_1	130	124-135
β_1	33	25-42
β^2	30	22-33
β_3	23	23-25

* These data are from 10 normal adult persons.

These findings of several SLAP components in normal persons are in direct contrast to those of Kowlessar(5), and Rutenburg(6), who report only one component in normal persons. The nature of these SLAP components and factors influencing their elaboration are being actively investigated.

Summary. Four distinct leucylaminopeptidase components have been found in normal human sera by DEAE Cellulose chromatography. In cases of hepatobiliary disease, there is apparently an increase in all components rather than the appearance of new components.

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Effects of Amine Oxidase Inhibition on *E. coli* Endotoxin Mortality in Mice.** (27222)

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The role of vasoactive amines in shock due to bacterial endotoxin has received increasing attention recently. The observation of Gordon and Lipton(1) that pretreatment of mice with serotonin (5-hydroxytryptamine) reduced endotoxin mortality suggested that inhibition of endogenous serotonin catabolism by inhibition of monoamine oxidase (MAO) might also be effective in protecting against endotoxin. Such an effect was demonstrated

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