SERUM LEUCINE AMINOPEPTIDASE IN CARCINOMA OF PANCREAS AND OTHER DISEASES

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Leucine aminopeptidase (L.A.P.) is an enzyme which acts preferentially on peptides in which the free amino group is that of an L-leucine residue. Its presence has been demonstrated in all human tissues assayed, with high activity in the duodenum, liver, and kidney (Green, Tsou, Bressler, and Seligman, 1955; Nachlas, Crawford, and Seligman, 1957). In 1958 Goldbarg and Rutenburg described a new, simple colorimetric method for the determination of this enzyme in the serum and urine, and reported high serum levels in pregnancy and in patients with metastatic malignant disease. Later the same year, they reported their observations on leucine aminopeptidase activity in the urine and serum of patients with cancer of the pancreas and other diseases (Rutenburg, Goldbarg, and Pineda, 1958). In this paper they showed that the serum level was greatly increased in all 12 patients with carcinoma of the head of the pancreas, and that two patients with carcinoma of the tail of the pancreas had smaller but nevertheless significant increases. They also claimed that obstructive jaundice due to stones in the common bile-duct produced rises in serum activity which were smaller and less persistent than those seen in carcinoma of the pancreas, and that primary liver disease also resulted in serum elevations in general lower than those seen in pancreatic cancer.

Because of the potential clinical importance of any investigation which might facilitate the earlier diagnosis of carcinoma of the pancreas, it was decided to try to confirm these observations. During the past nine months some 500 determinations of L.A.P. levels in human serum have been carried out. It is the purpose of this paper to present the results obtained.

Method

The assay method used was essentially that of Goldbarg and Rutenburg (1958). The substrate, L-leucyl β -naphthylamide hydrochloride, is incubated with serum in a phosphate buffer solution for two hours at 37°C. At the end of this time the reaction is stopped and the proteins are precipitated by the addition of trichloroacetic acid. After centrifuging, an aliquot of the supernatant is treated with sodium nitrite to accomplish diazotization of β -naphthylamine liberated by the enzyme. The addition of N-(1-naphthyl) ethylenediamine dihydrochloride then results in the development of a blue azo-dye colour which is proportional to the amount of β -naphthylamine liberated. The colour is stable for 24 hours at room temperature. The technical details of the method are given below as an appendix.

Units.—Rutenburg and Goldbarg expressed their results in terms of the Klett colorimeter reading obtained, whereas we have preferred to express ours in terms of the amount of β -naphthylamine liberated. For this purpose we have defined one unit of L.A.P. as that amount of enzyme which liberates one micromol of β -naphthylamine in two hours under the conditions of the test. We have then expressed our results as units of L.A.P. per 100 ml. serum.

The normal range was determined from a series of 107 subjects of all ages admitted to hospital without evidence of disease of the liver or biliary tract or of any other disease previously reported to give abnormal serum L.A.P. levels. For this group the mean serum L.A.P. was found to be 348 units/100 ml., with a standard deviation of 87 units. The normal range has been arbitrarily accepted as the mean $\pm$ twice the standard deviation—that is, 174–522 units/100 ml. The upper limit of normal has therefore been taken as 520 units/100 ml. While accepting that the upper limit of normal is lower for females than for males as reported by other workers, the difference has not seemed to us great enough to be of real importance in the clinical interpretation of the results obtained.

Results

Carcinoma of Pancreas

The serum L.A.P. has been studied in 16 cases of pancreatic carcinoma and the results are shown in Table I. The diagnosis was made in every case on the findings either at laparotomy or at necropsy.

TABLE I.—Serum L.A.P. in Patients with Carcinoma of Pancreas

No.	Site of Tumour	Bilirubin (mg./100 ml.)	Alkaline Phosphatase (K.A. Units/100 ml.)	L.A.P. (Units/100 ml.)
1	Head	12.6	38.0	2,480
2	"	8.2	—	1,560
3	"	16.0	—	1,245
4	"	10.3	45.5	1,150
5	"	10.0	76.2	1,070
6	"	16.0	28.1	1,028
7	"	13.9	27.1	960
8	"	†	—	891
9	"	12.3	35.8	680
10	"	†	—	635
11	"	<0.4	6.8	452
12	Neck	<0.4	—	264
13	Tail	2.4	34.2	647
14	"	<0.4	—	496
15*	Head	<0.4	—	430
16*	"	<0.4	16.1	870

* Samples were obtained from these patients only after cholecyst-jejunostomy.

† Serum icteric bilirubin level not known.

— No contemporary determination of alkaline phosphatase done.

Three patients had tumours arising in the neck or tail of the pancreas; two of these had no jaundice, and one, with multiple hepatic metastases and malignant ascites, was slightly jaundiced (serum bilirubin 2.4 mg./100 ml.). The remaining 13 patients had carcinoma of the head of the pancreas; of these, 10 were jaundiced. In two of the three remaining cases samples were obtained only some months after cholecyst-jejunostomy for the relief of obstructive jaundice (Cases 15 and 16).

The mean serum L.A.P. level for the 14 patients on whom the determination was made before any operative procedure was carried out was 970 units/100 ml. It will be shown that this level is no greater than that commonly seen in viral hepatitis or biliary obstruction due to stones. The enzyme level was normal in the one case of carcinoma of the head of

the pancreas without jaundice and in two of the three cases of carcinoma arising in the neck or tail, although one of these patients (Case 14) had a tumour the size of a grapefruit in the tail of the pancreas. Case 15, in whom blood was obtained for the first time two months after cholecyst-jejunostomy, had a normal serum L.A.P. despite the fact that the tumour was still *in situ*, and presumably progressing, as the patient died of this disease within six months. Case 16, studied three months after a bypass operation, had a raised level, but also showed a raised serum alkaline phosphatase (16.1 K.A. units/100 ml.), suggesting possible liver metastases. The only patient (Case 13) who had a carcinoma of the tail of the pancreas and showed an increased serum L.A.P. died within a week of the determination and had multiple hepatic metastases. It will be shown that the presence of secondary deposits in the liver could explain the rise in the L.A.P. seen in these two cases.

It has been possible to follow one patient (Case 7) by serial determination after cholecyst-jejunostomy, and the results obtained are shown in the chart. The serum L.A.P. level declined rapidly after operation and had fallen to 610 units/100 ml. in 22 days, at which point the patient was discharged to convalescence.

Serial determination of L.A.P. in a patient with carcinoma of the head of the pancreas before and after cholecyst-jejunostomy. The patient was discharged from hospital on the 22nd day, and no further follow-up sample could be obtained.

From these observations it must be concluded that the elevated levels of serum L.A.P. seen in many patients with carcinoma of the pancreas are probably the result of liver damage due either to extrahepatic biliary obstruction or to the presence of hepatic metastases rather than to the pancreatic tumour itself.

Other Pancreatic Diseases

Three cases of acute pancreatitis seen all had raised levels of L.A.P., with values of 1,500, 2,010, and 820 units/100 ml. respectively. The L.A.P. was normal in one case of subacute recurrent pancreatitis in which the serum amylase was moderately raised and in two cases diagnosed clinically as having chronic pancreatitis with pancreatic insufficiency. L.A.P. was also normal in each of 10 diabetic patients examined.

Diseases of Liver and Biliary Tract

The results obtained in 53 patients are shown in Table II. In all but two cases the diagnosis given was

TABLE II.—Serum L.A.P. Levels in 53 Cases of Liver Disease

Diagnosis	No. of Cases	Serum L.A.P. (Units/100 ml.)
Acute hepatocellular jaundice:		
Acute necrosis of liver ..	1	1,740
Subacute necrosis of liver ..	1	1,155
Infective hepatitis ..	8	680; 1,330; 1,334; 985; 1,075; 1,030; 800; 892
Hepatitis complicating infectious mononucleosis ..	1	727
Toxic hepatitis complicating chronic urinary infection ..	1	825
*Post-operative jaundice? cause ..	2	825; 888
Cirrhosis ..	12	495; 710; 1,000; 1,190; 1,070; 720; 675; 690; 392; 592; 650; 740
Intrahepatic obstruction:		
Chlorpromazine jaundice ..	1	1,260
Primary biliary cirrhosis ..	1	2,300
Extrahepatic obstruction:		
Stone in bile-duct ..	5	2,110; 1,070; 1,960; 1,105; 907
Inflammation of biliary tract:		
Acute cholecystitis ..	1	298
" cholangitis ..	1	645
Chronic cholecystitis ± cholelithiasis ..	4	340; 296; 398; 333
Hepatic malignant disease:		
Primary malignant hepatoma (arising in cirrhotic liver) ..	1	737
Hepatic metastases ..	5	1,716; 940; 475; 1,010; 685
Vascular:		
Chronic passive venous congestion ..	2	705; 444
Neonatal jaundice:		
Physiological jaundice ..	1	655
Haemolytic disease ..	3	595; 550; 470
Biliary atresia ..	1	3,010
Familial hyperbilirubinaemia ..	1	298

* One case after parotidectomy, the other after operation for aneurysm of the anterior cerebral artery.

either proved by histology, laparotomy, or necropsy, or the diagnosis was beyond reasonable doubt on clinical grounds. The two exceptions were cases of jaundice that followed operations on the cranium or face, in which there was no evidence of pre-existing liver disease and in which no wholly satisfactory diagnosis could be reached.

Patients with viral hepatitis in the icteric phase all showed raised serum levels of L.A.P. The mean value for the nine cases was 983 units/100 ml., which is of the same order as the mean level obtained in cases of pancreatic cancer. Values greater than 1,000 units/100 ml. were found in single cases of acute and subacute hepatic necrosis and of chlorpromazine jaundice.

The enzyme activity was increased in 10 out of 12 cases of cirrhosis examined, though the increase tended to be less than that seen in viral hepatitis, the mean value for the group being 744 units/100 ml. However, it exceeded 1,000 units/100 ml. in three cases. A marked rise was observed in a patient with primary biliary cirrhosis (2,300 units/100 ml.), and this observation has been confirmed on several occasions.

In five patients with cholelithiasis and obstructive jaundice the mean level was found to be 1,430 units, which is higher than the mean for all cases of carcinoma of the pancreas.

A patient with a malignant hepatoma arising in a cirrhotic liver showed a moderate increase (737 units), and a raised L.A.P. was found in four of five patients with proved hepatic metastases.

The patients examined with acute or chronic cholecystitis with or without cholelithiasis showed normal levels.

A baby with congenital biliary atresia showed the highest level seen in this series (3,010 units). The levels in four other neonates examined with physiological jaundice or jaundice due to haemolytic disease were only slightly above the normal adult limits and in no case exceeded 700 units.

Malignant Disease

The results obtained in 20 cases of proved malignant disease, other than carcinoma of the pancreas, are shown in Table III. Seven patients showed elevated

TABLE III.—Serum L.A.P. in 20 Cases of Carcinoma Other than Carcinoma of Pancreas

No.	Site of Tumour	Proved Hepatic Metastases	Serum L.A.P. (Units/100 ml.)
1	Breast	—	572
2	"	—	276
3	"	—	354
4	"	—	472
5	Bronchus	—	427
6	Pharynx	—	287
7	Oesophagus	—	545
8	Stomach	—	246
9	"	—	420
10	Ampulla of Vater ..	—	1,290
11	Colon	—	320
12	Sigmoid	—	464
13	Kidney	—	417
14	Bladder	—	286
15	"	—	417
16	Breast	+	1,716
17	Bronchus	+	940
18	Caecum	+	475
19	Colon	+	1,010
20	Rectum	+	685

+ Indicates proved hepatic metastases noted either at operation or at necropsy.

levels of L.A.P. Of these, four had proved liver metastases, one had a carcinoma of the ampulla of Vater with obstructive jaundice, and the other two had minimal rises only (572 and 545) and could possibly have had undetected liver secondaries. The case of carcinoma of the ampulla of Vater is of considerable interest, in that this patient presented with a clinical picture and operative findings indistinguishable from carcinoma of the head of the pancreas. The L.A.P. was greatly increased and fell rapidly after cholecyst-jejunostomy, regaining the normal level within three weeks. This case again emphasizes that biliary obstruction produces a rise in L.A.P., and that this rise is independent of the nature of the pathology producing the obstruction.

The remaining cases all had L.A.P. levels within the normal range.

Miscellaneous Disease

The serum L.A.P. has been determined in a wide range of miscellaneous conditions, but no significant abnormalities have been found. In particular it is perhaps worth recording that it is normal in most cases of myocardial infarction, though in patients who are in failure or are profoundly shocked there is sometimes a small rise possibly due to anoxaemic liver-cell damage. Renal failure with uraemia does not result in retention of the enzyme with elevated serum levels. Normal levels have been demonstrated in the reticuloses and collagen diseases without evidence of hepatic involvement.

Discussion

These findings are in agreement with those of Arst, Manning, and Delp (1959), who state that normal L.A.P. levels are found in a wide variety of malignant, neurological, cardiovascular, haematological, gastro-intestinal, renal, dermatological, and infective disorders, but that almost any condition which affects the liver may produce abnormal serum levels of the enzyme.

We have been unable to confirm the clinical usefulness of this determination for the diagnosis of carcinoma of the pancreas. The increases seen in this condition are no higher than those seen in viral

hepatitis or in jaundice due to intrahepatic or extrahepatic obstruction from other causes. Moreover, three cases reported here, which were not jaundiced and had no evidence of hepatic metastases, had normal L.A.P. levels—despite the fact that one patient with a carcinoma of the tail of the pancreas had a tumour the size of a grapefruit. After bypass operations for the relief of obstructive jaundice produced by pancreatic cancer, the L.A.P. can be shown to fall, although the tumour is still *in situ*. It must be concluded that any rise in serum L.A.P. which occurs in patients with pancreatic carcinoma is in no way directly related to the presence of the neoplasm.

The determination has little promise as an aid to the differential diagnosis of jaundice, since it is less effective than the commonly available tests in differentiating between jaundice due to extrahepatic obstruction and that due to hepatocellular disease.

The finding of a very high level in a baby with congenital biliary atresia does suggest a possible limited usefulness in clinical medicine. The diagnosis of extrahepatic obstruction in the neonatal period is notoriously difficult; the van den Bergh reaction is often negative owing to inefficient conjugation of bilirubin with glucuronic acid by the liver, and urobilinogen is normally absent from the urine in neonates owing to the absence of a bacterial flora in the gut. A test which could reliably detect obstruction might be of considerable value.

It has not been possible as yet to establish the normal neonatal range for serum L.A.P., but it seems unlikely that the high level found in the maternal serum in the last trimester of pregnancy crosses the placental barrier; and the upper limit of normal in babies is probably not much greater than that for adults. Results so far suggest only minor elevations of L.A.P. in physiological jaundice and jaundice due to Rh—or ABO-incompatibility. Possibly consideration of the L.A.P. level in conjunction with the isocitric dehydrogenase value (which in adults is normal in extrahepatic obstruction, but much raised in liver-cell necrosis) may permit earlier and more certain recognition of congenital biliary obstruction. It is, however, clear that much further work will be required before any conclusive evidence can be submitted on this point.

Summary

Serum leucine aminopeptidase (L.A.P.) may be determined for clinical purposes using L-leucyl β -naphthylamide hydrochloride as the substrate. The method is simple and satisfactory.

Raised serum levels are seen in pregnancy, acute pancreatitis, viral hepatitis, necrosis of the liver, chlorpromazine jaundice, liver metastases, extrahepatic biliary obstruction, and in some cases of cirrhosis.

There is no evidence that the determination is of any value as an aid to the diagnosis of carcinoma of the pancreas.

APPENDIX**Estimation of Leucine Aminopeptidase****Reagents**

- (1) L-leucyl β -naphthylamide hydrochloride solution, 0.4 mg./ml.
- (2) 0.2 M phosphate buffer, pH 7; 0.2 M Na_2HPO_4 , 6.1 parts; 0.2 M KH_2PO_4 , 3.9 parts.

Equal volumes of reagents 1 and 2 are mixed to yield a substrate-buffer solution, pH 7.1, stable at room temperature for 30 days.

(3) Trichloroacetic acid solution, 12%.

(4) Trichloroacetic acid solution, 40%.

(5) Sodium nitrite solution, 0.1%.

(6) Sodium sulphamate solution, 0.5%.

(7) N-(1-naphthyl) ethylenediamine dihydrochloride solution, 0.5 mg./ml. in 95% ethanol.

Method

1 ml. of a 1:50 (2%) dilution of serum in distilled water is added to 1 ml. of the substrate-buffer solution. A control is prepared by adding 1 ml. of distilled water to 1 ml. of substrate-buffer solution. The test and control are both incubated at 37° C. for two hours.

At the end of this time the reaction is stopped and the proteins are precipitated by the addition of 1 ml. of 12% trichloroacetic acid. After centrifuging, a 1-ml. aliquot of the supernatant is pipetted off and 0.2 ml. of 40% trichloroacetic acid is added, and mixed by gentle shaking. (The original method described for protein precipitation was found to be unsatisfactory in that it sometimes resulted in turbidity in the final solutions.)

1 ml. of 0.1% sodium nitrite solution is added. Leave for five minutes. Any excess nitrite is then destroyed by the addition of 0.8 ml. of 0.5% sodium sulphamate solution. This reaction is complete in three minutes.

2 ml. of N-(1-naphthyl) ethylenediamine dihydrochloride solution is then added. The maximum blue colour develops in 15 minutes, and after this the optical density (O.D.) is read against the control at 580 mμ.

The colour is stable for 24 hours at room temperature.

The O.D. reading is converted to terms of β-naphthylamine liberated by reference to a standard calibration curve prepared with β-naphthylamine. When an O.D. greater than 0.700 is obtained, the procedure is repeated using an incubation time of one hour only, and the result obtained is doubled.

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PSYCHOGENIC URINARY RETENTION AFTER PARTURITION, RESULTING IN HYDRONEPHROSIS

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Urinary retention in women may occur in association with a wide variety of disorders such as retroverted gravid uterus, haematocolpos, uterine fibroids, and ovarian or broad-ligament tumours. Occasionally it is the presenting symptom in multiple sclerosis. Again, it is a common feature of the immediate post-partum period when bladder tone is reduced and large quantities of retained urine can be tolerated without producing a strong desire to micturate. Bruising and oedema of the bladder neck, perineal lacerations, and sutures are also contributory factors, but normal micturition is usually re-established within a few days.

Nevertheless, in a substantial proportion of cases no organic cause can be found to explain the disturbance. Emmett, Hutchins, and McDonald (1950) studied 76 cases of urinary retention in women who had attended the Mayo Clinic, and in the majority were unable to demonstrate a definite causative factor. They examined microscopically tissue removed from the vesical neck during transurethral resection in each of these cases, and noted that fibrosis, inflammatory reaction, and glandular hyperplasia were not significant factors in the production of obstruction. They advocated transurethral resection of the bladder neck as the treatment of choice, and 81% of their patients had a favourable response.

In such cases where there is an incomplete understanding of the underlying mechanism in physical terms psychiatric evaluation would seem to be indicated. Many standard medical and surgical reference books comment briefly that urinary retention may occur as a hysterical symptom which usually responds to reassurance, although occasionally catheterization may be required. However, so far as can be ascertained there have been only two relevant psychiatric studies reported in the literature. Williams and Johnston (1956) demonstrated the primary importance of emotional factors in a 28-year-old woman suffering from recurrent retention. Psychotherapy resulted in elimination of the symptom. Chapman (1959) described a further case where the patient's symptom was related to sexual trauma, seduction, and rape by her stepfather. Psychotherapy was directed towards exploration of her hostility towards this relative, and it was noted that her episodes of retention occurred when her hostility threatened to become conscious. Again, psychotherapy led to the disappearance of the symptom.

This presentation outlines the psychological aspects of a further case where retention was on a hysterical basis. The term "hysteria" is employed here to denote a psychogenic condition where the symptoms presented by the patient are not motivated in full consciousness and are employed for the sake of some gain. Such an illness usually does not progress to the development of irreversible damage, although this may occasionally occur when protracted hysterical paralysis of a limb leads to muscular contractures. However, it must be almost unique for the patient to persist with a symptom

A World Health Organization Expert Committee on Mental Health, which has just ended a session in Geneva, decided that research on an international as well as a national scale is necessary if further advances are to be made in preventing mental illness. The Committee defined areas of priority for mental health research, placing high on the list researches into brain function, social attitudes, effect of cultural change, psychoses of the aged, effects of nutrition, and genetic factors. In its opinion the administration of mental health programmes should also be studied. (W.H.O./59.)