in the uterine preparation is indeed present in the serum of 95% of patients with cancer, as Makari claims.

Summary

A preliminary attempt to use the Schultz-Dale technique in the diagnosis of cancer has been successful in 23 cases of carcinoma, and unsuccessful in 26.

The claims of Makari and of Burrows that the test has an accuracy of over 95% have not been substantiated. On the other hand, the results can be taken to confirm the immunological basis of the method.

We are grateful to the consulting physicians and surgeons of the Royal Adelaide Hospital and of the Queen Elizabeth Hospital, Adelaide, who suggested that we should test serum from their cases; to Professor R. F. Whelan, of the University of Adelaide Department of Physiology, who gave us much assistance with the setting up of the Schultz-Dale baths and kymograph systems; and to Dr. J. Orde Poynton, Director of this Institute, for helpful interest and advice. This work was assisted by a grant from the National Health and Medical Research Council of Australia.

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THE SERUM LEUCINE AMINOPEPTIDASE TEST

AN APPRAISAL OF ITS VALUE IN DIAGNOSIS OF CARCINOMA OF PANCREAS

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Despite the multiplicity of laboratory tests of liver function the differential diagnosis of jaundice often presents a vexing problem. This is particularly true when carcinoma of the pancreas is considered, and clinicians would welcome any test which could confirm or exclude such a diagnosis prior to laparotomy.

From an annotation in the *British Medical Journal* (1958) it seemed that such a test was now available in the leucine aminopeptidase estimations as described by Rutenburg, Goldberg, and Pineda (1958). These writers claimed that it was possible to distinguish between patients with carcinoma of the pancreas and patients with other conditions which produce jaundice, by the measurement of the leucine aminopeptidase in serum and urine.

Their results could be summarized as follows:
(a) High serum and high urine values were obtained in all their patients with carcinoma of the pancreas (18 of

head; 2 of tail). (b) Other causes of obstructive jaundice gave high serum values, but not nearly so high as in carcinoma of the pancreas; the urine values sometimes showed a moderate rise. (c) There was a moderate rise, more so in serum than in urine, in other hepatic conditions—for example, cirrhosis, hepatitis, and cholecystitis. (d) Where hepatic metastases were present from primary growths outside the hepato-biliary tract, moderately raised serum levels were found.

This paper is an account of the attempt to confirm the diagnostic value of this test in a large series of patients (including 15 with carcinoma of the pancreas), most of whom suffered from diseases of the liver and bile-ducts.

Method

Leucine aminopeptidase is an enzyme which splits the peptide link between leucine and other amino-acids. It is not absolutely specific, as it can also act on any N-terminal amino-acid with an aliphatic side-chain. In man, it is found in the liver, kidney, spleen, skeletal muscle, alimentary tract mucosa and contents, brain, testis, pancreas, blood, and urine. Its function in the blood and urine is not known.

Each step in the method devised by Goldberg and Rutenburg (1958) was critically examined and verified; their method was finally adopted exactly. The principle of the method depends upon the enzymatic hydrolysis at 37° C. of a buffered substrate of L-leucyl- β -naphthylamine hydrochloride; the liberated β -naphthylamine is converted, by a modification of the Bratton and Marshall (1939) reaction, to a stable azo-dye which can then be estimated colorimetrically against a standard calibration curve prepared from β -naphthylamine hydrochloride. The test is easy to perform and needs no apparatus that will not be found in a routine clinical laboratory. The reagents are now readily available in this country. Serum can be stored at 4° C. for up to a month before analysis.

Goldberg and Rutenburg originally defined the serum activity arbitrarily in terms of the readings on a Klett colorimeter, and the urine activity in terms of the amount of β -naphthylamine liberated under the conditions of the test. As we intended to include renal clearance studies we decided to define our serum activity more specifically and in the same manner as for urine (a unit is the number of micrograms of β -naphthylamine liberated by 1 ml. of a 2% dilution of the serum). In practice, our unit is exactly 12 times as large as that of Goldberg and Rutenburg. These authors later redefined their unit for serum also in terms of β -naphthylamine liberated, and this new unit is also exactly one-twelfth of our unit (Goldberg, Pineda, and Rutenburg, 1959).

A few tests were carried out at the request of our clinical colleagues, but most of the results were obtained from the examination of jaundiced serum received for other investigations and of serum in which the clinical data of the request form indicated the possibility of a condition in which the leucine aminopeptidase might be changed. Fuller clinical information was obtained later from the case-notes or from personal meetings with the clinicians in charge. We discuss only those results where a firm diagnosis could be reached on clinical grounds, together with other laboratory investigations; all cases of carcinoma of the pancreas or secondary malignant deposits in the liver had had a laparotomy or necropsy.

Altogether 870 tests have been carried out—120 on 119 normal subjects and 750 on 552 patients.

Results

Our values for the serum leucine aminopeptidase (L.A.P.) levels in normal subjects are in good agreement with those of Rutenburg *et al.* (1958) (see Table). A result over 18 units was regarded as abnormal.

Normal Serum L.A.P. Values (Units)

Sex	No. of Subjects	Range	Mean	S.D.
A { Male ..	46	5.2-17.9	10.9	2.8
Female ..	73	4.5-19.3	10.3	2.9
B { Male ..	200	6.3-19.1	11.9	2.4
Female ..	60	6.7-17.5	10.8	2.3

A=Results obtained in this series. B=Results of Rutenburg *et al.* (1958), which have been converted from their "Klett" units to the units defined in this paper.

The results in conditions in which jaundice might be expected to occur are presented in Fig. 1. Each point refers to the value obtained at the first examination only, subsequent and serial estimations being excluded.

In our series of 15 patients with carcinoma of the pancreas, only one normal serum L.A.P. value was encountered. In this case no active treatment was possible, since laparotomy showed a tumour as large as a tennis ball in the head of the pancreas and many secondary deposits in the liver and elsewhere. Ten weeks later the L.A.P. level had increased from 9 to 15 units, the serum bilirubin from 0.6 to 1.3 mg./100 ml., and the serum alkaline phosphatase from 8 to 10 King-Armstrong units/100 ml. After another eight weeks, and three days before death, the levels had returned to 13 units, 0.6 mg., and 7.5 units respectively. It is worth recording at this point that in only one case of our series was a raised L.A.P. value found when the serum bilirubin was less than 1 mg./100 ml. This is unlike the experience of Rutenburg *et al.*, who indeed claim a special value for this test in the "anicteric patient." One patient had a carcinoma of the tail of the pancreas with hepatic metastases, and his serum

FIG. 1.—Results of serum L.A.P. values plotted as frequency distributions for the conditions investigated. Column 1=carcinoma of pancreas; 2=obstructive jaundice; 3=pancreatitis; 4=cirrhosis; 5=infective hepatitis; 6=acute cholecystitis; 7=primary cancer outside the hepato-biliary tract with hepatic metastases; 8=primary cancer outside the hepato-biliary tract without hepatic metastases. The broken line shows the upper limit of normal.

L.A.P. level at 25 units was raised slightly above the normal range.

All the cases of "obstructive jaundice" (column 2 in Fig. 1) were the result of cholecystitis and its sequelae, usually cholelithiasis. They include one case of intra-hepatic biliary-duct fibrosis after cholecystectomy, one of stenosis of the common bile-duct after cholangitis, and one of stenosis of the common bile-duct two years after surgery: the latter produced the highest serum leucine aminopeptidase value of our entire experience. The frequency scatter is seen to be the same as for carcinoma of the pancreas except that a higher proportion of results were within the normal range.

A similar scatter, without, however, reaching the highest levels of the first two groups, was observed in cases of pancreatitis, cirrhosis, infective hepatitis, acute cholecystitis, and primary cancer outside the hepato-biliary tract with hepatic metastases (columns 3, 4, 5, 6, and 7 respectively of Fig. 1).

Discussion

The problems of the differential diagnosis of jaundice (Reinhold, 1955) have been little affected by the introduction of the leucine aminopeptidase test. It has been especially disappointing that from our experiences we have been unable to support the statement of Rutenburg *et al.* (1958) that "assays of leucine aminopeptidase are especially definitive when normal in ruling out cancer of the head of the pancreas." It is also quite clear from the results presented in Fig. 1 that no distinction could be made by this test alone between carcinoma of the pancreas and other conditions which had given rise to jaundice. Though employing a different method of estimating the serum leucine aminopeptidase, the same conclusions had been reached by Fleisher, Butt, and Huizenga (1957): in 87 patients with obstructive jaundice they had only 62% of abnormally high values and there was no significant statistical difference among the results of three subgroups consisting of 26 cases with stone in the common bile-duct, 26 with stricture of the duct, and 35 with malignant tumours (exact sites not specified).

The main positive finding to be derived from Fig. 1 is the confirmation that there is a significant difference between patients with proved hepatic secondaries from non-pancreatic primaries (column 7) and those in whom secondaries could not be detected (column 8). Even here, however, in every instance where the serum L.A.P. was increased, so also was the established serum alkaline phosphatase test for biliary obstruction; in addition, the alkaline phosphatase could be increased in conjunction with a normal L.A.P. value.

It appeared that the serum L.A.P. test might be an indication of obstruction to the biliary flow, with no ability to distinguish between the various causes of obstruction. We decided to try to correlate our serum L.A.P. figures with those of serum bilirubin and serum alkaline phosphatase, estimated on the same sample, in patients with carcinoma of the pancreas and other obstructive jaundices.

The results showed a lack of any close correlation between the serum bilirubin and serum L.A.P. levels, thereby confirming the claim by Rutenburg *et al.* (1958) that there is no relationship between the serum L.A.P. level and the degree or duration of jaundice.

When, however, the serum alkaline phosphatase results were plotted against the serum L.A.P. values

FIG. 2.—(A) Correlation between the serum L.A.P. and serum alkaline phosphatase results in cases of carcinoma of pancreas and (B) in obstructive jaundice.

a reasonably satisfactory measure of correlation was found. This correlation was better for carcinoma of the pancreas (15 patients) than for "obstructive jaundice" (33 patients) (Fig. 2). Furthermore, the calculated regression lines suggested that with carcinoma of the pancreas the serum L.A.P. lags behind the rising serum alkaline phosphatase, as compared with cases of obstructive jaundice. This finding has little value in the diagnosis of any individual case.

The difference could arise in two ways. Firstly, it could be ascribed to a lowered renal threshold for leucine aminopeptidase in cases of carcinoma of the pancreas and thereby explain the higher urinary L.A.P. excretions recorded by Rutenburg *et al.* There is no obvious reason for such a change, and indeed our preliminary urinary studies suggested that generally the urinary L.A.P. level was merely a reflection of the serum L.A.P. level. The second possible explanation is that carcinoma of the pancreas usually leads to a persistent and ever-worsening degree of obstruction, while other causes of

obstructive jaundice are often remitting. It may be, therefore, that, as the unrelenting obstruction continues, the liver cells lose their ability to manufacture leucine aminopeptidase. (This theory is open to testing by the assay of enzyme in the liver cells and secretions in animals after the common bile-duct has been obstructed experimentally.) There seems to be no excess direct secretion of the leucine aminopeptidase by the pancreas itself, as a by-pass operation to relieve an obstruction, no matter its cause, leads to a progressive fall in the serum levels of the L.A.P. bilirubin, and alkaline (see Fig. 3).

Fig. 4 shows the remarkable degree of correlation which may be found in an individual patient (recurrent cholangitis) between the serum L.A.P. and serum alkaline phosphatase. However, the values may move in diverging directions; so far, it has not been possible to glean any additional information of use to

the clinician from a study of these apparent discrepancies.

The alkaline phosphatase was abnormal in numerous patients with hepato-biliary disease while the serum L.A.P. persisted within normal limits. In the reverse position, in the nine instances out of our 750 tests with a raised serum L.A.P. and a normal serum alkaline phosphatase, eight already had laboratory evidence of liver dysfunction with a raised serum bilirubin.

The original workers had included studies of the urinary excretion of leucine aminopeptidase. We have made relatively few of these tests because of the difficulties involved in the collection of a proper 24-hour specimen, the inconvenience of dialysis (found later to be unnecessary if a "blank" is deducted to correct for other chromogens), and the early discovery in one of our patients who was convalescent from a cholecystectomy for gall-stones, of an excretion of 2,100 units, which is three times the highest figure recorded by Rutenburg *et al.* in carcinoma of the pancreas.

FIG. 3.—Serial studies in patients with leather-bottle stomach and secondary deposits in glands at hilum of liver, to demonstrate the divergent changes in the serum L.A.P., bilirubin, and alkaline phosphatase.

FIG. 4.—Serial studies in a patient with recurrent cholangitis.

Summary

Results of 750 estimations of serum leucine aminopeptidase on 671 subjects do not support claims that a normal level would exclude the diagnosis of a carcinoma of the pancreas. In our hands the test did not distinguish between carcinoma of the pancreas and other causes of jaundice; it merely denoted the presence of an obstruction to the biliary flow, without reference to the cause of obstruction.

Only extremely rarely does this test yield information not already supplied by the established tests of serum bilirubin and serum alkaline phosphatase.

ADDENDUM.—Of six further patients with carcinoma of the pancreas, tested since the compilation of this paper, five had raised values and one was within normal range.

Our thanks are due to Dr. E. M. Darmady and to the Board of the Portsmouth and Isle of Wight Area Pathological Service for enabling us to obtain a supply of the substrate before it was available in this country. We have also to thank the many clinical colleagues who co-operated in this investigation.

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Medical Memoranda

Granulocytopenia after Treatment with Phenytoin Sodium

Phenytoin sodium ("epanutin") has been used in the treatment of epilepsy for 21 years (Merritt and Putnam, 1938). During this time numerous side-effects have been described—skin rashes, cerebellar incoordination, gastro-intestinal symptoms, hirsutism, and hyperplasia of the gums. Unlike the closely related drug methoin ("mesontoin"), it rarely causes suppression of the bone-marrow. Bernhardt (1950) has described granulocytopenia after treatment with phenytoin, but his patient was also receiving methoin, so that the responsibility for bone-marrow suppression does not necessarily lie with the former.

In view of the rarity of this important side-effect after the use of phenytoin in an epileptic patient, the following case is presented.

CASE REPORT

The patient, a man aged 34, was admitted to hospital with a history of frequent epileptic attacks for about a year. There were no abnormal physical signs, but arteriography demonstrated a small angioma in the Rolandic fissure on the left side. This was successfully removed at operation, without sacrifice of brain tissue. After the operation he was dysphasic and had a right hemiparesis, but these had improved considerably within a week. He had not received

any anticonvulsant therapy before admission, but after operation phenytoin, 100 mg., and phenobarbitone, 50 mg., were given six-hourly. After one week this regime was changed to eight-hourly administration of the same dose of the two drugs, and two weeks later he was discharged to convalescence with this anticonvulsant therapy.

He was readmitted 12 days later in status epilepticus. Intramuscular paraldehyde was given at frequent intervals to bring the fits under control, and phenytoin, 100 mg. three-hourly, and phenobarbitone, 100 mg. six-hourly. On the next day he received 400 mg. of phenytoin and 400 mg. of phenobarbitone, and this was continued till the fourth day after admission, when he complained of a sore throat. On examination he had a temperature of 101° F. (38.3° C.), angular stomatitis, hypertrophic gums, and a profuse growth of monilia over his palate, buccal mucosa, and gums. Haematology showed: haemoglobin, 78%; white-cell count, 2,500/c.mm.; neutrophils, 6%. Phenytoin was stopped immediately, and methoin and primidone ("mysoline") were given, the dose of the latter being increased over the next three weeks, while the methoin was reduced and finally discontinued. Phenobarbitone was continued unchanged. His monilial infection responded satisfactorily to nystatin lozenges. On the day after the cessation of phenytoin, his neutrophil count was 30/c.mm., with a platelet count of 280,000/c.mm. Twenty-four hours later his white-cell count had increased to 3,500, with neutrophils 32%, and bone-marrow examination showed normal regeneration. After a further two days his white-cell count was 7,750/c.mm., with neutrophils 75%, and subsequently remained within normal limits.

COMMENT

That phenytoin was responsible for the granulocytopenia in this patient is suggested by the rapidity with which his white-cell count returned to normal after the cessation of therapy with this drug. The condition was fortunately recognized before irreversible damage had been done to the bone-marrow.

Thirty-eight days elapsed between the beginning of treatment with phenytoin and the onset of symptoms due to granulocytopenia. The maximum daily dose that he received was 400 mg., and for more than three weeks the dose was only 300 mg. daily. The optimal dose-level is said to vary between 200 and 600 mg. daily (Goodman and Gilman, 1955). At no time, therefore, was he receiving a dosage that would be regarded as dangerous.

Methoin (5-ethyl-3-methyl-5-phenylhydantoin) has a well-marked depressant action upon the bone-marrow. Abbott and Schwab (1954) showed that in 23% of their 79 patients treated with it neutrophil counts fell below 2,500/c.mm. In view of the toxicity of this drug, it is surprising that phenytoin so rarely causes granulocytopenia. Its occurrence in this patient probably represents an uncommon constitutional sensitivity to this widely used anticonvulsant drug.

I thank Dr. Michael Kremer and Mr. Valentine Logue for their permission to publish this case.

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