Serum pepsinogen in the differentiation of megaloblastic anaemia

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SYNOPSIS Serum pepsinogen levels have been compared in 40 patients with severe megaloblastic anaemia with those obtained from 62 normal subjects, and with levels in 20 patients with pernicious anaemia in remission, 46 patients with iron-deficiency anaemia, and 18 patients with a duodenal ulcer.

The range in the normal subjects was 55 to 482 units per ml. It is suggested that a level of below 90 units per ml. should be regarded as indicative of gastric atrophy. It was found to be of value in the early differential diagnosis of severe megaloblastic anaemia. The estimation was found to be reproducible and can be carried out in a routine hospital laboratory.

The differential diagnosis of megaloblastic anaemia may require intensive laboratory and radiological investigations which are unsuitable for severely anaemic patients who require early treatment. Clinical examination of such patients may be of limited help and reliance has to be placed on laboratory procedures. In the time available it is not always possible to determine by microbiological assays whether the patient has a deficiency of vitamin B₁₂ or of folic acid. As in Britain the most frequent cause of megaloblastic anaemia is B₁₂ deficiency due to the stomach's failure to secrete intrinsic factor, Addisonian pernicious anaemia, tests of gastric function remain the most widely used methods of early differentiation. The demonstration of achlorhydria, as judged by the estimation of free hydrochloric acid in gastric juice following stimulation by histamine acid phosphate in doses of 0.01 mg. per kg. body weight have been shown to be unreliable (Kay, 1953), but the recommended augmented histamine test requires radiological facilities and can be an ordeal for the severely anaemic patient. Attention has therefore been given to the assessment of gastric function by the estimation of pepsinogen in plasma. It was shown by Mirsky, Futterman, and Kaplan (1952) that this level in patients with pernicious anaemia was below that of normal subjects, an observation since confirmed by others (Chinn, 1953; Hoar and Browning, 1956; Nolan, 1958). Various methods of estimating serum pepsinogen have been reported, that described by Edwards,

Jepson, and Wood (1960) being found by Bock, Arapakis, Witts, and Richards (1963) to be reproducible and reliable. We have therefore used this method to assess the value of serum pepsinogen estimation in the early differentiation of megaloblastic anaemias by comparing the levels in a group of such patients with those found in patients with pernicious anaemia in remission, iron-deficiency anaemia, duodenal ulcer, and with normal healthy subjects.

MATERIALS AND METHODS

NORMALS Serum pepsinogen was estimated in 30 healthy blood donors aged 20-61 years and in 32 hospital patients who had no form of gastro-intestinal, haematological, or renal disorder. These were all regarded as 'normal'.

MEGALOBlastic ANAEMIA This group consisted of 43 patients with macrocytic anaemia and megaloblastic erythropoiesis demonstrated by bone marrow biopsy. The cause of the anaemia was later determined by serum vitamin B₁₂ assay (*Euglena gracilis* z strain), serum folate assay (*L. casei*), ⁵⁸Co-labelled B₁₂ absorption tests (Schilling technique), folic acid absorption test (Chanarin, Anderson, and Mollin, 1958), faecal fat estimations on five-day stool collections, glucose tolerance curves, xylose absorption test, and the urinary excretion of formiminoglutamic acid (Figlu) following a loading oral dose of 15 g. of histidine. The level of gastric acidity after a dose of 0.5 mg. histamine acid phosphate was also determined.

ADDISONIAN PERNICIOUS ANAEMIA IN REMISSION Twenty-three patients who previously had macrocytic anaemia,

megaloblastic erythropoiesis, achlorhydria following 0.5 mg. histamine acid phosphate, serum vitamin B₁₂ levels below 100 $\mu\text{g}/\text{ml.}$, and had shown a complete response to vitamin B₁₂ therapy, were studied.

IRON-DEFICIENCY ANAEMIA This group consisted of 46 patients with hypochromic anaemia, a low M.C.H.C. and low levels of serum iron with raised iron-binding capacity. The iron deficiency was due to chronic haemorrhage from menorrhagia, haemorrhoids, epistaxis, or of nutritional origin. No patient known to have a peptic ulcer was included in this group.

DUODENAL ULCER Seventeen patients had radiological evidence of ulceration of the duodenum.

Pepsinogen was estimated by the method described by Edwards *et al.* (1960). 'Tyrosine-like' substances, released following incubation of test plasma at 37°C. and pH 2.0 for 24 hours using human plasma as substrate, were measured by reading the colour produced with Folin and Ciocalteu's reagent and sodium hydroxide in 1 cm. cells of an Optica spectrophotometer set at 680 m μ . The level of pepsinogen is expressed as units of 'tyrosine' liberated from the plasma substrate by 1 ml. of test plasma. For convenience the pepsinogen level in serum rather than plasma was determined in the present investigation, no difference being found in specimens from 10 normal subjects. For each estimation 2.5 ml. of serum was required and this could be stored frozen at -20°C., there being no loss of proteolytic activity in the 10 control specimens after such storage for six months.

RESULTS

NORMAL SUBJECTS The 30 blood donors showed a range of 55 to 413 units/ml. (mean 197 units/ml.). The 32 hospital patients without anaemia showed a range of 55-482 units/ml. (mean 196 units/ml.). As there is no difference between these groups they have been combined. Of the 62 subjects, 32 were males and 30 females, the males showing a range of 55 to 482 units/ml. (mean 202 units/ml. S.D. ± 128) and the females a range of 55 to 413 units/ml. (mean 189 units/ml. S.D. ± 129). There was no significant difference in level between sexes, nor did age influence the values.

The distribution of levels showed a positive skew curve (Fig. 1). The range for the whole group was 55-482 units/ml. (mean 196 units/ml. S.D. ± 103).

MEGALOBlastic ANAEMIA In 26 cases of Addisonian pernicious anaemia all had serum vitamin B₁₂ levels between 20 and 80 $\mu\text{g}/\text{ml.}$, no free hydrochloric acid following 0.5 mg. histamine, and subsequently they responded to vitamin B₁₂ therapy. In this group the serum pepsinogen range was 5-55 units/ml. (mean 34 units/ml. S.D. ± 19). In 15 cases of nutritional megaloblastic anaemia there was a deficiency of folic acid in 14, demonstrated either by low serum

FIG. 1. Distribution of serum pepsinogen levels in normal subjects.

folate levels or excretion of Figlu following 15 histidine. In three patients there were low serum B₁₂ levels, in association with the folic acid deficiency. No patient showed any evidence of intestinal malabsorption by the tests used. In each case there was complete response to treatment with either folic acid or vitamin B₁₂ and no relapse has occurred. All ate poorly of foods which contain folic acid conjugates such as green vegetables, meat, and dairy products. In five cases of this group there was no free hydrochloric acid in the gastric juice following 0.5 mg. histamine. The serum pepsinogen levels of patients in this group varied from 82 to 370 units/ml. (mean 183 units/ml.). Two cases of intestinal malabsorption had steatorrhoea and deficiency of folic acid with poor absorption following an oral dose of 3 mg. folic acid. The diagnosis was confirmed radiologically and both responded to a gluten-free diet and oral folic acid therapy. The serum pepsinogen levels were 115 and 360 units/ml.

ADDISONIAN PERNICIOUS ANAEMIA IN REMISSION The serum pepsinogen levels in this previously diagnosed and treated group of 23 patients varied from 13 to 70 units/ml. (mean 36 units/ml. S.D. ± 17).

IRON-DEFICIENCY ANAEMIA All 46 patients had levels of serum pepsinogen within the range of the normal subjects, the figures varying from 96 to 400 units/ml. (mean 160 units/ml. S.D. ± 58).

DUODENAL ULCER The range of serum pepsinogen in these 17 patients was 215-670 units/ml. (mean 340 units/ml. S.D. ± 117). In eight of these patients the estimation was made on serum collected before and after maximum body doses of histamine as recom-

FIG. 2. Comparison of groups of patients with pernicious anaemia in relapse and in remission.

mended by Kay (1953). No difference between levels was found.

Comparing the groups of patients with pernicious anaemia, those in relapse and those in remission (Fig. 2), no significant difference was found between the range or the mean values of serum pepsinogen. The difference between this combined group of 49 patients, where the range was 5-75 units/ml. (mean

35 units/ml. S.D. ± 19) and the normal subjects was highly significant ($P < 0.001$). Grouping all the patients with non-Addisonian megaloblastic anaemia, both nutritional and malabsorption defects (Fig. 3), the range was 82-370 units/ml. (mean 190 units/ml. S.D. ± 86) and a highly significant difference was found when compared with the combined group with pernicious anaemia ($P < 0.001$).

DISCUSSION

The physiological variations of plasma and serum pepsinogen were extensively studied by Mirsky *et al.* (1952) and by Chinn (1953). They found the levels to be fairly constant from hour to hour and from day to day in any one individual, there being no significant variation after food or following gastric stimulation by histamine or insulin, so a simple random sample is therefore characteristic of any individual. Except when gastric secretion is reduced a positive correlation exists between the serum pepsinogen level and the secretion of gastric acid and pepsin, high serum levels occurring in patients with duodenal ulcers (van Goidsenhoven, Wilkoff, and Kirsner, 1958). With reduced gastric secretion, such as occurs in patients with pernicious anaemia, atrophic gastritis, following partial gastrectomy and total gastrectomy, a measurable amount of pepsinogen is still present in serum (Hoar and Browning, 1956; Spiro, Ryan, and Jones, 1956; Nolan, 1958; Bock *et al.*, 1963). This may be due to the presence of gastric glands in the oesophagus or to the secretion of cathepsin from the glands of the intestinal mucosa.

The normal range of serum pepsinogen has been found to vary from 55 to 482 units/ml., which is in agreement with that quoted by Bock and his colleagues (1963). Due to differences in method and in the manner of expressing results they cannot be compared with the ranges reported by other investigators. No variation with age or sex was found in this series but slightly higher levels in males were recorded by Mirsky and his co-workers (1952) and by van Goidsenhoven and his associates (1958). Higher levels have also been found in infants during the first week of life (Grayzel, Elkan, Moghazeh, Schneck, and Garza, 1962) and a rise with age up to the third decade was reported by Mirsky *et al.* (1952). High levels have been found in patients with duodenal ulcers (Mirsky *et al.*, 1952; Chinn, 1953) in association with azotaemia (Hirschowitz, 1955) and in those receiving phenylbutazone (Muirden, 1961). In the present investigation the group of patients with duodenal ulcers had a mean level of 347 units per ml. compared with that of 202 units per ml. for the normal subjects. Comparison of the

FIG. 3. Comparison of serum pepsinogen levels in normal subjects with those in patients with various disorders.

ranges in these two groups (Fig. 3) shows, however, such a wide overlap that the estimation becomes of limited value in the diagnosis of duodenal ulcer. It is possible that the apparently normal persons with such high pepsinogen levels have a predisposition to duodenal ulceration.

The overlap of range between patients with pernicious anaemia and normal subjects is small, 86% of such cases in this series having subnormal pepsinogen levels. Bock and his colleagues (1963) showed that no patient with histological evidence of gastric atrophy had a serum pepsinogen level above 90 units per ml. The upper limit of 75 units per ml. for patients with pernicious anaemia in the present series is in agreement with this finding. It would appear that any person with a serum pepsinogen level below 90 units per ml. is likely to have gastric atrophy even though it be asymptomatic, and a level of over 90 units per ml. makes the diagnosis of pernicious anaemia unlikely. As the estimation can be completed in 24 hours it has a place in the early diagnosis of pernicious anaemia and was found by us to be more reliable than assessment of achlorhydria as judged by the absence of hydrochloric acid in gastric juice following the injection of 0.5 mg. of histamine. Being an indirect method of diagnosis, occasional misleading results may occur, so it would be of interest to compare its reliability with that of the assay technique for intrinsic factor as described by Abels, Bouma, and Nieweg (1963). This, however, requires gastric intubation which is particularly un-

pleasant for patients with severe anaemia. The Schilling B₁₂ absorption test requires the use of radioactive substances whereas the only special equipment used in pepsinogen estimations is photoelectric absorption meter. It can therefore be carried out in most routine hospital laboratories, the sample of serum being collected at the same time as that for microbiological assays.

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