1958

Studies of blood pepsinogen

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STUDIES OF BLOOD PEPSINOGEN

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Submitted in Part'al Fulfillment of the Requirements
for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1958

Omaha, Nebraska

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INTRODUCTION

A method for measuring gastric activity without intubation has long been desirable because gastric intubation is very distasteful to many patients. One answer seemed to be measurement of urinary pepsinogen levels which were shown to be elevated with duodenal ulcers and lowered in such conditions as carcinoma of the stomach and pernic ous anemia. About 1950 Mirsky *et al.*[3] adapted Anson's method of assay of pepsin[2] to measure pepsinogen levels in serum in order to eliminate kidney factors which sometimes falsely alter pepsinogen level in the urine. This paper will review all but three unavailable articles (including one in Czechoslovakian and one in Hungarian) on various studies of blood levels of pepsinogen and will present some original determinations and observations.

REVIEW OF LITERATURE

The following is the method of Mirsky for determining blood pepsinogen[4]. All other investigators either use this method or modify it slightly (the method used for the original work in this paper is a modification).

> 2 ml. plasma from oxalated blood is mixed with 10 ml. of 2.5% hemoglobin substrate, and the mixture adjusted to the pH desired with 3N HCl. Dilute to 15 ml. with water. Incubate a 6 ml ali-quot at 37° C. for 24 hours; then add 10 ml. of .3N trichloroacetic acid and filter. Do the same with an unincubated 6 ml. aliquot. A "blank" solution of distilled water and hemoglobin is treated like the plasma-hemoglobin mixture and likewise in-cubated 24 hours. Color is developed by adding 2 ml. of each filtrate (with its tyrosine-like products) to 3 ml. .3N trichloroacetic acid, 10 ml. 0.5N NaOH, and 3 ml. of diluted Folin-Ciocalteu reagent[1] (one part reagent and two parts water). Read O.D. on a spectrophotometer.

Although Mirsky uses a second blank composed of hemoglobin solution and water incubated at 37° C. (*vide supra*) to take into account any increase in optical density due to nonenzymatic splitting of the hemoglobin with the incubation, Hoar and Browning[20], Hirschowitz[10], Chinn[6], and Spiro *et al.*[9] do not do so. Various authors read optical density at 280 mu.[6], 420 mu.[20], and 560 mu.[10]

Mirsky *et al* [4] state that there are at least three enzymes with proteolytic activity assayed in normal

plasma. The first is active at pH 1.5-3.0. Most of this activity is lost after total gastrectomy or in the presence of pernicious anemia. This first one has the properties ofpepsinogen which is autocatalytically converted to pepsin at pH 1.5. The second is active at pH 1.5 and is apparently derived from tissues other than the stomach. The third is active at pH 1.5-3.0. Evidence of the third is seen in the presence of a relatively small but persistent (15-30%) activity in this pH range after gastrectomy or in the plasma of normal subjects after alkalinization of a previously acidified plasma, a treatment which destroys pepsinogen. The third was not further identified, but Mirsky thought that it must not have its source in the gastric mucosa. Spiro[19] suggested that it might be due to gastric glands of the esophagus or cathepsin from the small intestine (unlikely, as noted above) or other acid "proteases".

Hirschowitz[10] states that the third enzyme of Mirsky is really nonenzymatic splitting of proteins of the added plasma by hydrochloric acid used in the assay, however. He offers the following as proof: The hydrolysis of acidified hemoglobin substrate was taken into account with the blank. A sample with low activi-

ty was activated at pH 1.5 and divided into three parts--one was irradiated with ultraviolet light; one was alkalinized to pH 11, then reacidified (inactivated plasma); the third was analyzed as such. The three fractions were assayed for 24 hours by the standard procedure of Mirsky at 0°, 10°, 25°, 37°, 42°, 56°, and 72° C. Simultaneously the hemoglobin substrate was incubated with one milliliter of water at the same temperatures and pH to provide a blank for the inactivated plasma which would in turn serve as a blank for the activated plasma. The proteolysis of the activated plasma from which the proteolysis of the inactivated plasma is subtracted reacts to temperature changes as would be expected from a solution of pepsin. In the inactivated plasma the increase of tyrosine-like products parallels that of the nonenzymatic breakdown of the hemoglobin, and the difference in the two curves represents the nonenzymatic lysis of the protein in the plasma and the activity ascribed by Mirsky to a second proteolytic enzyme in the pH 1.5-3.0 range. The ultraviolet light treated plasma showed normal true peptic activity, but the protein was half again as susceptible as pH inactivated plasma to nonenzymatic acid hydrolysis at 70° C.

Mirsky[4] further states that since pepsin is inactivated rapidly at the pH of blood and since intravenous administration of pepsin in dogs does not increase the proteolytic activity of urin at pH 1.5, probably the major portion of the proteolytic activity of the plasma at pH 1.5-3.0 is due to the autocatalytic conversion of pepsinogen to pepsin at the low pH. Furthermore, intravenous administration of pepsinogen in dogs produced an increased proteolytic activity of the blood plasma at pH 1.5 and of urine at pH 1.5.

Hirschowitz[10] also offers certain experiments showing that the enzyme assayed for has an activity like pepsinogen and hence is almost certainly this enzyme precursor and not pepsin or any other proteolytic enzyme. No decrease in assayable peptic activity was produced by first alkalinizing to pH 11 and then returning the solution to pH 2 for assay; pepsin would have been destroyed by this procedure. While boiling destroyed all activity, 70° C. for 10 minutes did not destroy any. Ultraviolet light (3660 Å) did not decrease the peptic activity. Acidified (activated) plasma showed decreased peptic activity between 42° C. and 56° C. and no activity above 56° C. and also after alkalinization above pH 8. All of these characteris-

tics are consistent with the known behavior of pepsinogen.

Euglobulin was extracted from plasma[6]. In contrast to its known proteolytic activity at a higher pH it produced no hydrolysis of protein at pH 2.0. Euglobulin neither added to nor detracted from the proteolytic activity of whole serum at pH 2.0.

To determine if an inhibitor of pepsin might play a role in the activity of plasma at pH 1.5, Mirsky[4] added crystalline pepsin to acidified plasmas of known activity. 90% of the added pepsin was recovered, indicating that inhibitors play an insignificant role.

Pepsinogen was found in the white blood cells, red blood cells, and serum[10], with concentrations decreasing in that order. Serum concentration was the same as plasma concentraion[6,10]. The absolute amounts in white and red blood cells are small, and the stomach is responsible for all but a minute fraction in plasma and urine. One should be careful not to hemolyze the blood, however.

There was a significant correlation between mean hourly gastric pepsin output and the plasma pepsinogen concentration in 18 patients who were studied with simultaneous intubation and plasma pepsinogen determina-

tions[10]. No correlation with the concentration of gastric pepsin was obtained, however, because pepsin in gastric juice never exceeds a certain maximal concentration. Statistical studies of the correlation between plasma pepsinogen and mean hourly gastric pepsin showed that r=+.603, P=less than .001.

At this point it should be noted that actual figures given by the various authors for plasma or serum pepsinogen levels will not be given. Each laboratory has different normal values. Also, the pepsinogen levels are reported in several different units which are derived by different calculations from the optical density of the solutions. In several cases, however, the statistical correlations will be given because these figures do not depend on the type of units but rather on the differences between the groups.

The coefficient of variation in this procedure as shown by simultaneously running duplicated procedures on the same serum samples is 4-9%[10,20]. The coefficient of variation of daily determinations on the serum of the same person is 10-18%[5, 9, 19, 20]. One author[23] had one patient who had a much greater variation over a seven day period. The coefficient of variation of determinations done on serum of the same person at

various times during a single day is 8-10%[5, 20]. Variations during the day were completely unrelated to meals. Good reproducibility was obtained after refrigerating the plasma for two days[9] or after freezing it for up to one year[20].

In Mirsky's series of normal patients[5] the plasma pepsinogen increased slightly with increasing age, but Spiro's smaller series[9] showed a slight decrease with increasing age after age 30. Although the concentration of pepsinogen in the male tended to be greater than the female this was not statistically significant except in the 70-79 age group (P=.02-.05)[5].

Plasma pepsinogen determinations were first done to study various clinical conditions in which gastric secretion had been known to be altered and which are commonly studied by tube gastric analysis. Some of the results of the various authors are listed in Table I. In addition, it is interesting to note that Mirsky has shown[3] that patients with ulcerlike symptoms but without demonstrable duodenal or gastric ulcer by X-ray have pepsinogen levels in their serum similar to those found in patients with duodenal ulcers; whereas Meena et al.[23] state that these patients have plasma pepsinogen levels slightly below normal. All gastric ulcers in Table I are benign.

TABLE I--Comparison of blood pepsinogen levels of various groups

Comparison made by author	No. of reference	statistical correlation (author's)
duodenal ulcer group high than control group	20	P less than .01
	5	P less than .01
	6	
	7	
	9	
	10	P=.001-.0001
gastric ulcer group higher than control group	3	
	9	
	10	
	20	
duodenal ulcer group higher than gastric ulcer group	3	
	9	
	10	P=.01-.05
duodenal ulcer group approx. same as gastric ulcer group	20	
Both duodenal ulcer and gastric ulcer groups higher than gastric carcinoma group	9	
	11	P less than .001
	20	
massive gastrointestinal hemorrhage with duodenal ulcer higher than without duodenal ulcer	9	
esophageal stricture with duodenal ulcer higher than wi hout uodenal ulcer	9	
hiatus hernia with duodenal ulcer higher than without duodenal ulcer	9	
Subtotal gastrectomy with duodenal ulcer higher than without duodenal ulcer	9	
control group highdr than both subtotal and total gastrectomy groups	20	P less than .01
control group higher than gastric carcinoma group	20	
control group lower than gastric carcinoma group	9	
control group higher than pernicious anemia group	3	
	5	P less than .001
	6	
	20	

As noted in Table I there is good statistical separation of most of the various groups. This does not answer the question whether the test is a useful one, however. The standard deviation of the various groups is generally quite large so that there is a considerable degree of overlapping of individual values of the different groups. For instance, in Mirsky's series[11] although 50% of gastric carcinoma cases had plasma pepsinogen values lower than the lowest ulcer patient, 15% had values above the mean of the ulcer group. This test may be an adjunctive aid in differentiating gastric carcinoma from gastric ulcer in the 50%, however. Spiro, on the other hand concludes that this test is of no help in distinguishing these two entities because his series had slightly more overlapping[9].

Also, 87% of patients with duodenal ulcer have plasma pepsinogen levels above the mean of values found in subjects without duodenal ulcers or other gastrointestinal disturbances[5]. These values persist after the ulcer has healed. 14% of patients without gastrointestinal disturbances have values above mean values for patients with duodenal ulcers. When these "normal" patients develop a duodenal ulcer the plasma pepsinogen does not rise further. Note that mean values are men-

tioned rather than the top or bottom values. The determination of plasma pepsinogen is, therefore, more helpful in the exclusion of a duodenal ulcer than the proving of one[19]. Although a tendency for higher values was noted among patients with active lesion, it was not statistically significant[5].

The level of blood pepsinogen helps in the differentiation of cause of massive hematemesis, i.e., duodenal ulcer from other causes (e.g., esophageal varices)[9, 19]. Also, if anacidity is found in gastric juice, a high blobd pepsinogen suggests acid will be found on careful search, or this is a temporary case of anacidity. In some patients with a lot of mucus and bile buffering the gastric contents blood pepsinogen may be a better measure than free acid or a fall in pH.

Though there seems to be some doubt as to the usefulness of this test for the individual case under most circumstances, there is good agreement that the test is useful as a screening procedure and as a research tool for measuring gastric secretory activity in various groups or in the same group before and after a given procedure. Several such research studies will be reviewed below.

Sievers and Fischer[29] have compared tubeless gastric analysis (use of cation exchange resin with an indicator substance which is measured in the urine) and plasma pepsinogen determinations as screening procedures. Azure resin method took 1½-4 minutes per test, and quinium resin method took 8 minutes per test. Though they did not say how long the plasma pepsinogen test took, this author's own experience indicated that time per test would depend somewhat upon the number of determinations which could be run simultaneously but would probably be slightly longer than the resin tests in any case. 217 patients were used in the comparative study. The results are not strictly comparable, however, because the resin tests are on an all or none basis whereas the pepsinogen tests represent a continuum; also, the resins measure the acid production of the parietal cells whereas pepsinogen levels measure the production of the chief cells. Those with initially negative tubeless gastric analysis also proved achlorhydric with intubation and histamine in 56% and hypochlorhydric in an additional 25%. Usually achlorhydric patients had low plasma pepsinogen, and those with high plasma pepsinogen secreted free acid; but the deviation from "normal" of the acid and pepsinogen re-

sults was not necessarily in the same direction as is consistent with the fact that different cells are responsible for each secretion. About 80% of the achlorhydrics were below the mean of the controls for plasma pepsinogen. No conclusions were drawn as to which is a better test. The authors merely stated that these two indirect tests of gastric secretory function complement each other. Meena *et* al.[23] found no correlation between free or total acid and pepsinogen (plasma) when determined simultaneously.

A number of factors have been noted to affect plasma pepsinogen levels in addition to the gastric conditions listed above. There is an elevation of plasma pepsinogen levels in azotemia due to lack of normal kidney excretion of the pepsinogen (15 patients)[19]. Ephedrine (6 patients) caused a mean decrease in plasma pepsinogen while pilocarpine (5 patients) and chlorpromazine (2 patients) caused an increase[23]. Diabetic patients using depot insulin have higher levels of plasma pepsinogen than normal[19, 29] except in the presence of anacidity[29]. Patients (6) with acute myocardial infarction also had increased plasma pepsinogen levels[19].

Varro *et* al.[15] experimented with adrenalin and

cincophen. Adrenalin injection did not change plasma pepsinogen levels. It has been shown that long term administration o cincophen produces peptic ulcers in dogs by an unknown mechanism in about 20 days. In 11 dogs there was a gradual increase in plasma pepsinogen to a peak at 14-15 days and then a decrease until it was back to norm l at 21 days. The levels decreased for 10 days afte. cincophen was stopped followed by a rise to normal. 17-ketosteroids roughly paralleled the plasma pepsinogen which may indicate that the changes which occurred reflected changes in the functional capacity of the adrenal cortex during cincophen treatment.

Cooper et al.[24] tested both uropepsinogen and plasma pepsinogen in a group of 20 males, aged 22-65 (average34), all cigarette smokers who were free of gastrointestinal disease and had a normal urinalysis and blood urea nitrogen. They were tested again a week after they quit smoking. Stopping smoking produced a slight decrease in urinary pepsinogen which was not statistically significant; however, there was also a slight decrease in plasma pepsinogen which was statistically significant ($P<.003$)

Croog[25] determined plasma pepsinogen in 1016 army inductees of average age 21, 42% from New York City,

and less than 2% from rural areas. The Irish had significantly higher serum pepsinogen levels than Germans, Italians, and Jews (all of whom were approximately the same). Negroes fell in between these two groups (somewhat closer to the latter) but were not statistically significantly below the Irish. Among the Irish there was no difference between those with four grandparents from Ireland and those with one or more grandparents born in a countr other than Ireland. Also, there was no difference between those with both parents from Ireland compared with those with both parents from the United States. No significant relation was found between blood pepsinogen and occupation, education, occupation of fathdr, social class of father, or occupational ambitions. He concluded that the Irish have certain traits in common more than any other group in the sample which caused an increased serum pepsinogen.

The relation between plasma pepsinogen levels and stress or treatment with ACTH or adrenal corticoids is of great interest because of the occasional production of peptic ulcers with these factors. Hoar and Browning found a 25% increase in plasma pepsinogen in 10 patients after treatment with ACTH or cortisone for one week[20].

Kowalewski et al.[17] experimented with 20 dogs--5

controls, 5 hypophysectomized, 5 thyroidectomized, and 5 bilaterally ad enalectomized and maintained on cortisone. ACTH was given after the dogs had all stabilized; plasma pepsinogen was markedly increased in all but the adrenalectomized dogs. This paper will be discussed further when the effects of histamine are discussed.

Varro et al [15] determined plasma pepsinogen before and 1, 2, 5, and 6 hours after an ACTH injection in 24 normal patients and in 7 Addisonians. In 23 of the normals the single dose of ACTH produced a 14-49% increase in plasma pepsinogen at the peak (generally in the first 120 minutes and returned to the original level at the sixth hour. In the Addisonians there was no increase in the pepsinogen, and 4 had a significant decrease through an unknown mechanism.

Hirschowitz et al.[12] studied 5 normal male students, 19-25, with negative upper gastrointestinal X-rays who were given 25 u. ACTH every 12 hours for 6 days. An adequate adrenal response was indicated in all by increased 7-hydroxysteroid excretion and a decrease in the total eosinophil count. In all of them there was an increase in urinary pepsinogen but no change in plasma pepsinogen. They also had one patient

with a chronic duodenal ulcer who developed an acute ulcer in the second part of the duodenum. The plasma pepsinogen, which had been low, increased five times on the fifth day after symptoms started (the eighth day after ACTH therapy had been started). Plasma pepsinogen only gradually declined and was still in the ulcer range 34 days after X-rays showed healing. After nine months plasma pepsinogen was normal.

In another study Hirschowitz et al.[27] studied the effect of eitht hour infusions of ACTH, hydrocortisone, corticosterone, prednisolone, aldosterone, and histamine. ACTH, hydrocortisone, corticosterone produced an increase in urinary pepsinogen but no change in plasma pepsinogen. Aldosterone decrease urinary but not plasma pepsinogen. Changes in urinary pepsinogen corresponded to changes in urinary clearance of pepsinogen; these changes did not reflect similar changes in gastric secretion of pepsin. The authors concluded that ACTH works on the kidney via glucocorticoids which regulate urinary pepsinogen excretion (17-hydroxysteroids in the urine varies directly with urinary pepsinogen).

Shapiro et al.[21] determined blood pepsinogen before and after gi ing ACTH to 3 cats, 3 dogs, 10 rats, 9 healthy humans, 1 patient with pernicious anemia, and

1 patient with renal disease in an acute study. There was no rise or fall in any of them of significance. Also, blood pepsin gen was determined following chronic therapy in one patient with an inactive peptic ulcer and severe pulmonary disease and in four without ulcers. There was no significant change in any of them except that the ulcer patient had a transient rise for the first week. They concluded that in instances of adrenal hormone ulcers the hormone probably plays a permissive rather than a causal role and operates by increasing the sensitivity of the gut to stimuli previously not damaging, possibly due to direct tissue changes, vascular disturbances, or changes in the character of the mucus secretion of the stomach.

Belkin and Shapiro [6] exposed rats to a stressful situation which had been previously shown to produce behavioral disturbances. One group got distilled water, the other 1.5% saline to drink. There was no significant difference in plasma pepsinogen in either group as compared with nonstressed controls after ten weeks. Also, no ulcers were produced in stomach or duodenum.

Shapiro and Horn[14] produced an experimental neurosis in cats with the Masserman techniques. A hunger-fear conflict was induced. The large majority of the

cats did not develop a significant change in blood pressure. Changes in blood pepsinogen were of meager significance and showed a downward trend. There was an inverse relationship between blood pressure and pepsinogen level in the blood. No ulcerations were noted in stomach or duodenum of sacrificied cats.

Because of the known effects of histamine on gastric secretion a number of experiments have been done investigating its effect on plasma pepsinogen. Hoar and Browning[20] did not find increased plasma pepsinogen in dogs in whom ulcers were created with histamine ir in Ivy pouches with antral transplants. This finding is in marked contrast to those to be reviewed below.

Kowalewski and co-workers have conducted several studies with histamine[8, 13, 17, 18, 28]. In the first of these[8] ten guinea pigs received very high doses of histamine with promethazine (to permit giving a dose of histamine 1000 times the normal lethal dose). Gastric ulcers were prod ced as was shown when they were sacrificied four hours later. There was a significant increase in plasma pepsinogen as compared with ten non-treated animals which served as controls. In later studies with guinea pigs[28] gastric juice pepsin and pepsinogen in homogenized gastric tissue as well as plasma

pepsinogen were measured. Histamine was given as before (75 mg. per kg. body weight), and the animals were sacrificied 1, 2, 3, and 4 hours later (12 guinea pigs in each group). Histamine produced a significant rise of pepsinogen (gastric tissue and blood) and pepsin (gastric juice) in all animals. Though the experiments permit this conclusion only for exogenous histamins, it is known that gastric mucosa is rich in histamine which may be extracted and used as a gastric stimulant.

In further experiments[13] dogs were treated with promethazine and histamine. Plasma pepsinogen was measured before and 90 and 180 minutes after histamine and before histamine but after anesthesia. Anesthesia with pentobarbital pr duced a slight decrease in plasma pepsinogen as is consistent with the slightly reduced gastric activity generally produced by barbiturates. Three dogs which received 2.0 mg. histamine per kg. body weight had 32-72% increase in plasma pepsinogen. Three dogs receiving 4.0 mg. per kg. has 74-124% increase in plasma pepsinogen. Six dogs receiving 5.0 mg. per kg. had 142-223% increase in plasma pepsinogen.

Eight dogs were given 5 mg. histamine per kg. and an antihistaminic as in other experiments[18]. Plasma pepsinogen determinations were done on specimens from

gastric vein, gastric artery, and cephalic vein. Blood was taken after laparotomy by cannulation, one hour la-ter when an antihistaminic was injected, followed in 30 minutes by histamine, and 1, 2, 3, and 4 hours after histamine. Gast ic juice was aspirated at intervals by a Levine tube. No change was noted before and after operation before histamine was given. Histamine produced a significant increase in plasma pepsinogen in all animals with a maximal rise 3-4 hours after the administration of the histamine. The ratios of plasma pepsinogen concentrations noted were as follows:

gastric vein:gastric artery=1.20-1.27 before histamine
gastric vein:gastric artery=1.35-1.44 after histamine
gastric artery:cephalic vein=1.14 before histamine
gastric artery:cephalic vein=1.07-1.09 after histamine

In three dogs in whom laparotomy was done 3½ hours af-ter histamine the following ratios of concentrations of plasma pepsinogen were obtained:

portal vein:hepatic vein=1.10
hepatic vein:gastric artery=1.11
gastric vein:gastric artery=1.7

The increase in plasma pepsinogen roughly paralleled the increase in gastric pepsin during the first two hours after histamine after which plasma pepsinogen continued increasing and gastric pepsin decreased. It was con-

cluded that the liver does not alter pepsinogen because the differences between hepatic and portal veins are no greater than can reasonably be explained on the basis of dilution by blood from the hepatic artery.

Using the same dogs as were used in determining the effects of ACTH (vide supra), Kowalewski et al.[17] determined the effects of histamine. All dogs had a significant increase in plasma pepsinogen with histamine. Control dogs responded better than hypophysectomized and thyroidectomized dogs, but the most marked increase was in the adrenalectomized dogs (four of whom died and had erosions of the gastric mucosa). They concluded that histamine raises plasma pepsinogen by acting directly on zymogenic cells and not via ACTH. They also suggested that adrenalectomized animals may have decreased tissue histaminase. These results in adrenalectomized dogs are opposite to those listed previously for Addisonian patients[15]; the reason is unclear.

Earle et al.[26] demonstrated the effect of gluca-gon and glucose on plasma pepsinogen. In five dogs injected with 200 micrograms of glucagon intravenously a peak elevation in blood sugar was noted in 15-30 minutes. A fall in blood pepsinogen levels was noted in all cases which was significant (P less than .02). Five other dogs given a slow intravenous glucagon infusion over

2-4 hours at 2. -3.3 micrograms per minute showed a significant fall in blood pepsinogen in every case during the period of glucagon induced hypoglycemia and for an hour after the blood sugar was back to normal. Three animals given an intravenous infusion of glucose showed a significant fall in blood pepsinogen comparable to that seen with glucagon administration. Two of the three humans given glucagon had a significant decrease in blood pepsinogen. These results and the observations that insulin increases blood pepsinogen[19, 29] indicate that the blood glucose is an important factor in the secretion of plasma pepsinogen. Also, the above experiments refute glucagon as the cause of a recently described clinical triad of duodenal ulcer, gastric hypersecretion, and pancreatic islet cell tumor (Zollinger and Ellison, Ann. Surg., 142:709, 1955) and suggest insulin as the possible pancreatic ulcerogenic factor.

Weiner, Mirsky, et al.[22] state that there are three parameters which may contribute to the precipitation of a duodenal ulcer: a physiological parameter, which determines the susceptability of the duodenum to ulceration; a specific psychic conflict that induces psychic tension; and a social parameter, which determines the environmental event that will prove noxious to the

particular individual. They determined blood pepsinogen on 2073 draftees, 17.5-29.2 years old, from northeastern United States. 63 of the top 15% and 57 of the lowest 9% were given a battery of psychological tests without the examiners knowing their pepsinogen level, and an upper gastrointestinal X-ray series was run. All but 13 were again given psychological tests and X-rays after 8-16 weeks of basic training. The first X-rays showed three healed duodenal ulcers and one active duodenal ulcer, all among the gastric hypersecretors as measured by plasma pepsinogen. The second X-rays showed acti e ulcers in five more, all hypersecretors. Criteria were looked for in the psychological tests which have been shown to be common in patients with duodenal ulcers, e. g., intense infantile oral dependent wishes, "immaturity", tendency to please and placate, and difficulties revolving particularly around management of oral impulses and hostility. Other criteria were looked for which were found in hyposecretors. A psychologist and two psychiatrists independently rated the test records using these criteria. On the basis of majority opinion 71% of the hypersecretors and 51% of the hypdsecretors were correctly designated. Using a cluster of 20 criteria, each of which

had been shown o be accurate to the 5% level of confidence, it was possible to distinguish the two groups to the extent that 85% of the 120 could be accurately assigned to their group at a .001 level of confidence (no individual criteria were greater than 65% accurate). It was impossible to determine why only some of the hypersecretors reacted as they did to the social situation or developed the duodenal lesion. The study indicates that neither a high rate of gastric secretion as measured by plasma pepsinogen nor a specific psychodynamic constell tion is independently responsible for the development of a peptic ulcer.

The following is a discussion of the author's own experience and determinations with the blood pepsinogen test.

METHOD

I. 1. Place 1 ml. serum in a calibrated test tube.

2. Add 5 ml. of 2.5% aqueous bovine hemoglobin solution.

3. Adjust mixture to pH of approximately 2.0 (1.5-2.5) by adding an appropriate amount of 1.5N HCl (generally about .3 ml.)

4. Dilute to 7.5 ml. by addition of an appropriate amount of water.

II. 1. Prepare a blank by taking a 3 ml. aliquot from I.

2. Add 5 ml. .3N trichloroacetic acid.

3. Filter through Whatman No. 3 filter paper.

4. Freeze 2 ml. of the filtrate in a test tube large enough to hold at least 16 ml.

III. 1. Incubate the remaining mixture from I. at 37° C. for 24 hours as the unknown sample.

2. After 24 hours take a 3 ml. aliquot.

3. Add 5 ml. .3N trichloroacetic acid.

4. Filter through Whatman No. 3 filter paper.

5. Place 2 ml. of the filtrate in a test tube as was done with the blank.

IV. 1. To 2 ml. of each filtrate (unknown and blank) add the following:

a. 3 ml. .3N trichloroacetic acid

b. 10 ml. .5N NaOH

c. 1 ml. undiluted Folin-Ciocalteu Phenol Reagent

V. 1. Read optical density of the unknown sample at 310 mu. on a Beckman du Spectrophotometer with the machine set at 0 with the blank after the mixtures have been sitting for 5-10 minutes.

For the sake of convenience serum samples were frozen until time could be found to run them. As noted in the previous discussion there is good reproducibility after being frozen for as long as one year. In addition, a large amount of serum was obtained from one patient, and serum pepsinogen was determined immediately.

Other aliquots of the same serum were frozen, and determinations were made at six different times over the following month. The optical density of the various determinations only varied from .160 to .169.

The chemical rationale of the test is as follows. The pepsinogen is autocatalytically converted to pepsin at pH 2.0. The cathepsin type of plasma protease is inactive at this pH. The hemoglobin provides a protein substrate for the pepsin to digest to release tyrosine-like products. Maximal digestion occurs at approximately 37° C. Trichloroacetic acid is used to prepare a protein-free filtrate. Sodium hydroxide produces a markedly basic solution, which is necessary for the reaction of the Folin-Ciocalteu reagent which reacts with the tyrosine-like products released upon the digestion of the hemoglobin to form a bluish complex which can be quantitated by the use of a spectrophotometer.

RESULTS

The patients in this study are divided into an adult group with the youngest adult being 19 and into a childhood group with the oldest child being 16. Scatter diagrams show the results graphically--see Fig-

ure 1 for adults and Figure 2 for children. In addition, Tables II and II summarize adults and children respectively and the results of the statistical analysis of the figures obtained[30,31,32]. For all groups the mean, standard deviation, and standard error of the group will be given. The means of the groups will be compared by use of Fisher's calculations and Table of t. It is especially desirable and significant to use Fisher's t when comparing small groups (less than 50). Also, the variances of the groups will be copared by use of Snedecor's calculations and Table of F. The differences in variances is a less important test and merely measures a difference in homogeneity of groups.

It is noted that the gastric ulcer group and pernicious anemia group differ from the adult controls in the direction that would be consistent with the results found by other investigators as listed previously in Table I. The results were not significant statistically, however, probably because the groups are so small. The gastric carcinoma group is also equivocal as is also consistent with reports in the literature.

As was noted previously, there is disagreement concerning the effect of age on pepsinogen levels. In

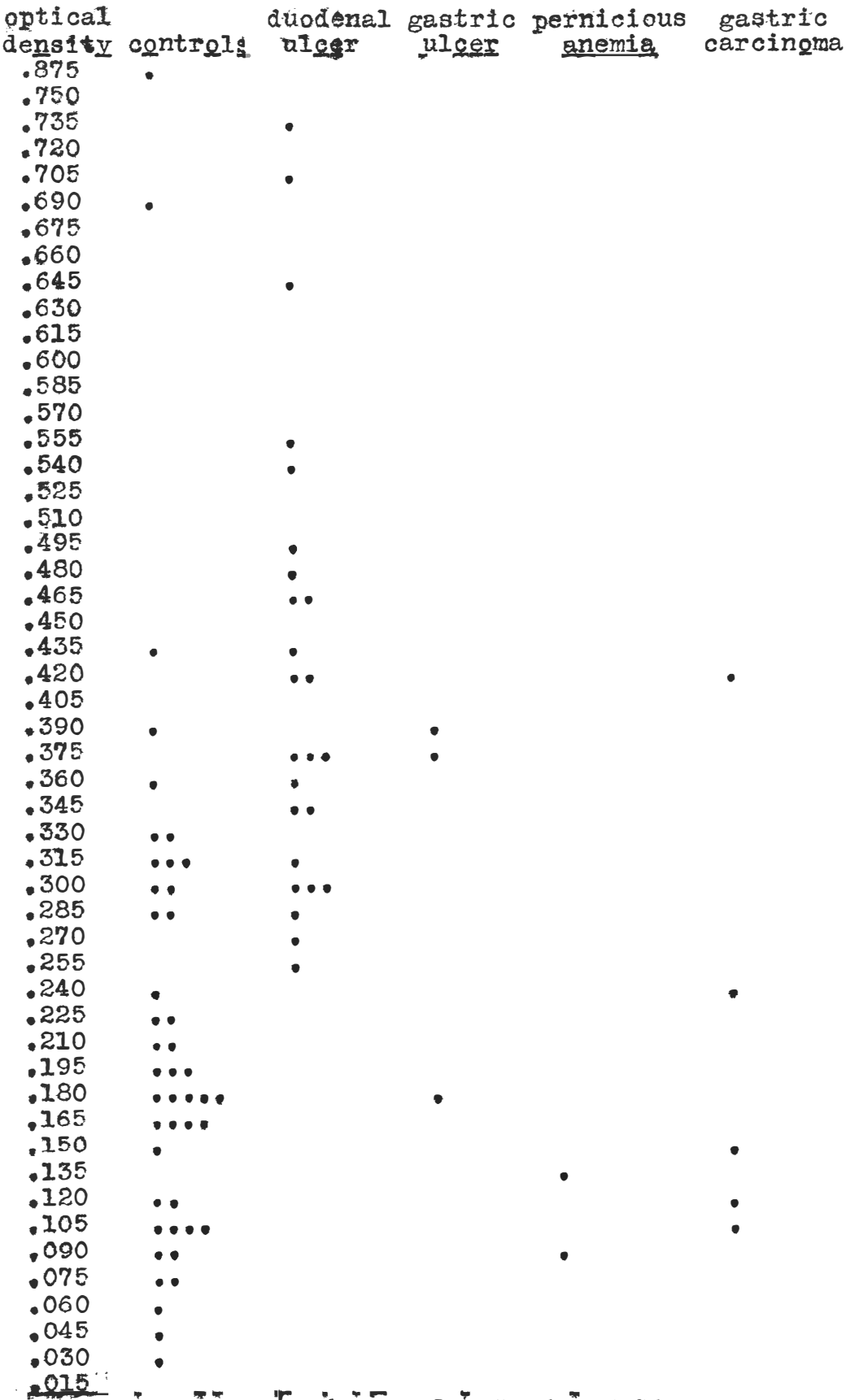

Figure 1--Serum pepsinogen levels in adults

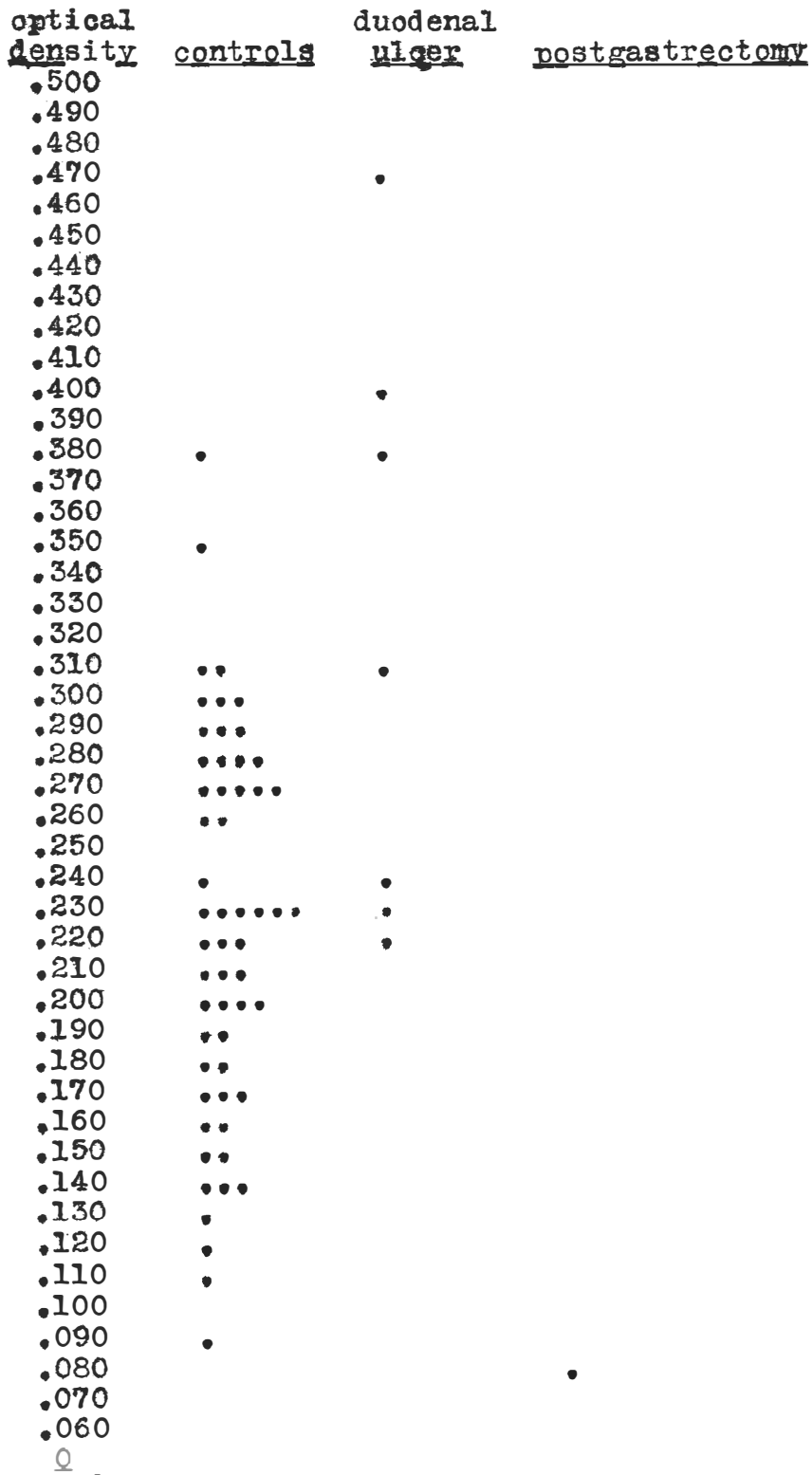

Figure 2--Serum pepsinogen levels in children

TABLE II--Serum pepsinogen in adults, statistical correlation

group	mean	std. dev.	std. error	compare to	t	F	P
controls	.222	±.158	.024	---	--	---	---
duodenal ulcer	.422	±.132	.026	controls	5.8		less than .001
				controls		1.43	greater than .05
gastric carcinoma	.204	±.138	.062	controls	.15		greater than .05
				controls		.76	greater than .05
gastric ulcer	.313	±.124	.072	controls	.4		greater than .05
				controls		1.6	greater than .05
pernicious anemia	.115	±.036	.025	controls	1.2		greater than .05
				controls		20.	greater than .05

TABLE III--Serum pepsinogen in children, statistical correlation

group	mean	std. dev.	std. error	compare to	t	F	P
controls	.224	±.063	.008	---	--	---	---
duodenal ulcer	.321	±.097	.037	controls	3.6		less than .001
				controls		2.35	less than .05

this series controls were the same in adults and children.

Duodenal ulcer groups were higher than controls in both adults and children. The differences were statistically significant. There was a certain degree of overlapping of groups as has been noted by all other investigators.

Several isolated cases of interest were obtained. One steroid induced ulcer had O. D.=.370, which falls within the duodenal ulcer range. One marginal ulcer following a Billroth I partial gastrectomy had O. D.=.305. A patient with a lymphoma of the stomach had O. D.=.030, a very low value. No statistical correlations can be done with single cases.

DISCUSSION

The primary purpose of this paper is to present serum pepsinogen levels in children in order to determine if they are elevated in the presence of duodenal ulcer as had been previously noted in adults. Only one other case of duodenal ulcer in childhood has been reported[5]. Seven additional cases are presented. The results are highly significant statistically (see Table III). This may be evidence for a common etiology for

duodenal ulcers in both adults and children at least in so far as gastric physiology is the determining factor of ulceration. The fact that adult ulcer patients produced results similar to those reported in the literature adds weight to the results herein reported for the child ulcer patients because it shows that the test was performed properly.

It should be noted that the groups in childhood are much more homogeneous in makeup than in adults, i.e., the standard deviation is much smaller. This can probably be explained by at least two factors associated with aging. Some of the lower values in adults may be due to diminished gastric secretory function which has been noted with aging, e.g., the per cent of patients with achlorhydria increases with increasing age; these patients are included in the control series if they do not have any other proven gastric disease. Also, there may have been patients included in the control series of adults who had changes in their kidneys associated with aging and affecting renal clearance of pepsinogen but who did not yet have clinical uremia. Neither gastric analysis nor N.P.N. were determined in the control series. Nor was it considered desirable to do so. In order to have greater clinical significance the control

series should include a normal hospital population. Not even patients with gastrointestinal diseases other than those studied were eliminated from the studies though most other authors have done so. The only patients not included in the control series were those in uremia and those who used insulin because of the marked effects which these factors may have on the blood pepsinogen as has been shown by other authors.

The use of this test as a diagnostic procedure is somewhat limited as can be seen by inspection of Figures 1 and 2. The highest value obtained was in one of the control patients, who had toxemia of pregnancy; unfortunately an N.P.N. was not obtained on her. In the upper ulcer ranges the test is fairly specific, but at least one author[1] has noted that patients with ulcer symptoms but without an ulcer will have blood pepsinogen levels within the ulcer range. Thus, in patients with symptoms this is not a good test for differential diagnosis. This can be a good test for the exclusion of an ulcer, however, if the level is low, at least in adults. The same is probably true in children, but the series was small for children so that dogmatic statements should be avoided.

As a screening procedure this would probably be an

adequate test. The author's experience would indicate that it would take slightly longer per test than the length of time others noted for the quinium resin method and considerably longer than the time for the Azure A resin test[29]. It would have the advantage that samples could be obtained on an assemblyline basis but the disadvantage that special personnel would be needed to collect the samples. It would also be advantageous in that there would be no problem of having the patients take the resin at the proper time before specimens were obtained. Probably the balance would weigh against serum pepsinogens.

The test is perfectly adequate as a research tool as a measure of gastric activity. Individual values may not always be helpful, but groups of values can be compared with significance by the use of the proper statistical tests.

SUMMARY

1. Mirsky's method for determining plasma or serum pepsinogen is presented.

2. This method is shown to measure pepsinogen and possibly a second blood protease, although values for the latter may be simply nonenzymatic acid hydrolysis

of the plasma.

3. Mean hourly gastric pepsin output and plasma pepsinogen concentrations were correlated.

4. Normal variations in plasma pepsinogen are presented. Variations were small.

5. Various authors have shown elevated levels in duodenal and gastric peptic ulcers and decreased levels following gastrectomy and with pernicious anemia. There were equivocal results in gastric carcinoma.

6. This method is compared with the use of tubeless gastric analysis without any definite conclusions being reached.

7. Cigarette smoking was shown to elevate plasma pepsinogen.

8. Ethnic background was of significance in the Irish who had higher levels than Germans, Italians, and Jews.

9. Effects of ACTH on plasma pepsinogen were equivocal, some reporting an elevation and some no change.

10. Stress did not significantly change the plasma pepsinogen.

11. Histamine was an effective stimulus for increased plasma pepsinogen in all except one paper.

12. The liver did not affect plasma pepsinogen.

13. Glucagon was noted to decrease plasma pepsinogen asdid intravenous glucose.

14. An overall concept of duodenal ulcerogenesis with the significance of physiological factors is presented.

15. A modification of Mirsky's method of analysis is presented which was used in determining original data presented.

16. Chemistry involved in the test is discussed.

17. Data for adults is shown to be comparable to that obtained by other authors.

18. Original data concerning children with duodenal ulcers is presented, and significant elevation of serum pepsinogen is shown.

19. An evaluation of the test from the practical standpoint is presented. Its main use will probably remain in the field of research.

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