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UROPEPSIN DETERMINATION AS A MEASURE OF GASTRIC SECRETION

BY

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HISTORICAL BACKGROUND

In 1861, mention was first made of an enzyme with proteolytic activity which was present in normal urine.[12] It was postulated that either the enzyme was pepsin and derived from the intestine by absorption, or that it was a material secreted directly into the blood stream by the gastric mucosa.[13] The source of this enzyme was demonstrated by Frouin in 1904, who showed that it disappeared from the urine of gastrectomized dogs and that it remained in the urine when the stomach of a dog was externalized by a pouch technique. Because of this latter finding, he concluded that the enzyme had a gastric origin and was absorbed by the stomach rather than the intestine.[20]

Gottlieb, in 1924, concluded that the urinary enzyme was pepsinogen,[22] although prior to and since that time it has been referred to as uropepsin[13] (most common usage), uropepsinogen,[31] urinary pepsinogen,[29] or merely urinary peptic-like activity. The enzyme will be referred to as uropepsin in this paper.

The first clinical application of uropepsin determinations was made by Farnsworth, Speer, and Alt when they demonstrated low values of uropepsin in patients

pernicious anemia.[19] Since this study, much as appeared in the literature concerning the possible value of uropepsin studies as an adjunct in the clinical evaluation of other diseases involving the stomach, especially as a method of distinguishing between peptic ulcers and gastric neoplasm. However, many factors operate to affect the level of uropepsin excretion, and these will be considered in the following pages.

PHYSIOLOGY AND CHARACTERISTICS

Although there has been disagreement in the literature concerning even such basic facts as the effect of histamine, anti-cholinergic drugs, and elevated gastric hydrochloric acid levels upon uropepsin excretion, and whether such determinations accurately reflect gastric pepsin levels or not, the following general concepts concerning the physiology of gastric and urinary pepsin become evident upon reviewing the literature.

Under the influence of ACTH, adrenal cortical hormones stimulate the chief cells of the gastric mucosa to secrete pepsinogen.[45] It has been postulated that other gastric stimuli such as vagal stimulation, histamine, alcohol, caffeine and gastrin operate primarily by increasing parietal cell secretion (acid) and therefore have little effect upon pepsinogen secretion.[6,48]

This theory is significant in view of the fact that histamine induced acid production has no effect on uropepsin values.[3,40] There is disagreement in this area, however. One author reports a linear relationship between HCl concentration and pepsin after histamine with a correlation between pepsin and uropepsin.[1] Another, however, reports increased levels of both HCl and pepsin secretion, but no correlation of uropepsin with either of these values, concluding that uropepsin instead reflects the mass of normal gastric tissue rather than the state of secretion.[48] Evidence has been recently brought forth, however, demonstrating the possibility of a catheptic enzyme produced by the stomach which is also excreted in the urine and exhibits maximum activity at a different pH than uropepsin. Histamine did not result in parallel stimulation of excretion of these two enzymes,[35] and this fact may explain the variable results reported by different investigators.

The pepsinogen which is secreted by the stimulated chief cells is partitioned into exocrine and endocrine portions, approximately 99% being secreted into the stomach and converted by acid to pepsin, and 1% being secreted into the blood stream.[31] The pepsinogen entering the gastric lumen is assumed not to be reabsorbed because of its rapid conversion to pepsin, which has been shown not to be absorbed from the stomach or intestine even when fed

orally in large amounts.[36] Therefore the pepsinogen circulating in normal plasma is the result of endocrine peptic activity.

Several theories have been advanced concerning methods of transport of pepsinogen in the bloodstream. Because there is detectable pepsinogen in plasma, transport as free pepsinogen is possible. In 1926, however, a pepsin-inhibitor complex was described which was thermolabile at 55° Centigrade, undialyzable, and extractable in ether. Animals with high uropepsin activity had high levels of inhibitor complex.[13]

Before studies on the excretion of pepsinogen by the kidney had been made, it was generally thought that glomerular filtration occurred, followed by partial tubular reabsorption.[29] A recent study demonstrates a linear relationship between creatinine clearance and pepsinogen excretion in ten subjects. From their data, the authors have concluded that pepsinogen is excreted as a non-threshold substance by glomerular filtration and that either tubular absorption or excretion does not occur, or the tubule absorbs a constant percentage of pepsinogen. However, because of a molecular weight of forty-two thousand the authors feel that tubular reabsorption is unlikely.[27]

As it finally appears in urine, the enzyme is

stable at room temperature for four days if the pH remains stable and there is no bacterial turbidity, and stable under refrigeration for 15 days.[13] Bucher, in a review article,[12] stated that maximum activity occurred at pH 2.0 - 3.4, but most investigators employ a pH of 1.5. Because inactivation does not occur in neutral solution[19] it was felt that the enzyme was present in urine as pepsinogen rather than pepsin which is inactivated at pH 6.0 - 9.0.

Refrigeration without toluene retains the highest activity according to some authors,[21] but others feel that toluene does not effect activity and insures stability.[36] There is no evidence of a low molecular weight inhibitor in urine because dialysis and washing of urine with chloroform failed to alter activity, and addition of urine to pepsin did not alter the peptic activity.[36]

METHODS OF DETERMINING UROPEPSIN

Several techniques have been described for the determination of uropepsin, all involving enzymatic digestion of a substrate by the activated enzyme in acidified urine. The basis for preference of one technique over another depends on the experience and consistency of results obtained by the individual investigator. The

technique described by Anson and Mirsky[2] and variations of it is probably most commonly used. Because it is the technique which was used by the author in determinations described subsequently, it will be described in detail.

Method of Anson and Mirsky[2]

A 20 ml. aliquot of urine is taken from a twenty-four hour collection of urine, adjusted to pH 1.5 with concentrated HCl, and made up to a total volume of 25 ml with distilled water.

One ml of the acidified urine and 5 ml of substrate (acid hemoglobin solution) are placed in separate tubes into a water bath maintained at 37° and allowed to incubate for five minutes. Then the 5 ml of warmed substrate is added to the 1 ml of urine and the mixture is incubated for 30 minutes. At the end of this time the reaction is stopped by addition of 10 ml .3 N trichloracetic acid. As a control, 1 ml of acidified urine and 5 ml of substrate are incubated in separate tubes for 35 minutes, at the end of which time they are added together and the reaction is stopped immediately with trichloracetic acid.

The contents of each tube are filtered through Whatman #50 and the filtrate is retained. For the colorimetric determination, the following are added to a 50 cc Erlenmeyer flask in order: 2 ml of filtrate, 12 ml distilled water, 8 ml .5 N NaOH, and 3 ml of

Folin-Ciocalteu reagent (diluted 1 part reagent to 7 parts water). After 30 minutes of color development, readings are made in a colorimeter using a filter with wave length 540. The difference between the experimental and control readings is interpreted as mg tyrosine released, employing a standard tyrosine curve.

The hemoglobin substrate is prepared by initially making a 2½% aqueous solution of commercial hemoglobin. Because it is unstable the acid hemoglobin substrate is prepared daily by adding 20 ml of .3 N HCl to 80 ml of 2.5% hgb solution. Larger or smaller amounts of acid substrate can of course be prepared with appropriate dilutions.

The results of the determination are expressed as milligrams of "tyrosine-like substances released by the proteolytic activity (uropepsin) of 1 ml of urine incubated with 5 ml 2½% hemoglobin substrate at 37° C for 30 minutes. The total uropeptic activity for a 24-hour period is arrived at by multiplying the results by the volume in milliliters of the 24-hour sample. This figure would be expressed as milligrams of tyrosine released by a 24-hour sample of urine. To avoid such an unwieldy form of expression, uropeptic units have been defined.

Hirschowitz defines a unit as that amount of enzyme which will release the spectrophotometric

equivalent of 1 x 10^{-4} meq of tyrosine from .1 g of hemoglobin mixture. (This equals .0181 mg of tyrosine)[29] Gray et al define 1 unit as that amount of enzyme which will release .04 mg tyrosine-like substance during 30 minutes of incubation.[24]

Method of West[47]

The technique reported by West involves the conversion of casein in milk to paracasein in acetate buffer at a pH of 4.9. 1 ml of urine is incubated for 45 minutes with .05 ml 2 N HCl which brings the incubation pH to 3.0. At the end of incubation .8 ml of distilled water, 1.0 ml pH 4.9 acetate buffer and .5 ml of equal parts homogenized milk and acetate buffer are added to .20 ml of activated urine. The end point is reached when granules of paracasein are seen to be precipitated and this time is recorded. If the reaction requires more than 200 seconds, a larger portion of activated urine is mixed with substrate for the next determination. One unit of uropepsin with this technique is defined as the number of ml of activated urine x 10 required to give an end point in 100 seconds. This is equivalent to .26 Armour crystalline pepsin. Units per hour may also be determined by the following equation:

Units / hr = 1/10 x V/vh (100/t) 1.32 where

v = volume in ml
t = seconds until end point is reached
V = total volume
h = hours during which V was excreted.

A graphic method of determining units of uropepsin is available with this technique which is much simpler for clinical use.

Method of Christensen

The most recent technique reported is that of Christensen[14] who uses edestin as a substrate which is incubated with diluted urine for 2 hours at 30° C. The reaction is then stopped with 5 cc of 10% trichloracetic acid. The supernatant is decanted, the precipitate is washed and redissolved in 35% urea. The absorbancy of this solution at 280 mu is determined against a blank of 35% urea. A standard of Edestin and urine precipitated with trichloracetic acid and redissolved in 35% urea is also used. The technique is felt by Christensen to be valuable because it is not interfered with by the presence of chromogenic substances which are present in urine and do not exert absorbant effects at the wave length employed.

UROPEPSIN EXCRETION IN NORMAL INDIVIDUALS

In reading the literature concerning uropepsin it is important to appreciate the different definitions of units of uropeptic activity. This becomes important when

one is confronted by the "normal" values established by different investigators. Gray et al,[25] in a control group of 265 patients, established a mean value of 3670 units with a standard error of / 159 units for a 24-hour period. Goodman et al[21] using a series of 29 controls with the West method established a mean of 28 units per hour with a standard deviation of 14.5%.

The excretion is fairly constant in one individual when daily determinations are made, the standard deviation in one series not exceeding 12%.[45] Because there is variation in excretion from hour to hour, most workers in the field collect 24-hour samples. The rate of excretion is not affected by the volume, acidity or specific gravity of the urine nor is it affected by physical status such as amount of sleep or moderate exercise. An early report mentioned a high protein diet as increasing uropepsin excretion,[13] but this has been refuted by more recent work.[11,37] One subject normally on a diet containing 70 grams of protein showed no change when this was increased to 150 grams per day.[21] Uropepsin levels are higher in smokers than in non smokers.[8]

Sex - Sex is felt to play no part in the excretion of uropepsin in normal individuals,[4,44] although two studies report higher values in males than in females.[10,18]

Age - Normal individuals in the preadolescent group

have lower values than those of adults[10] but after age 50, 65% of normal subjects have decreased uropepsin values.[34] This would seem to correlate with the prevalence of achlorhydria in the aged. One study reports that 28% of normal females and 23% of normal males are achlorhydric at age 60.[46] These figures would indicate decreased total gastric function in the aged, rather than simply a deficiency of gastric acid.

Menstruation - Menstruation and pregnancy both increase the excretion of uropepsin.[4]

STRESS AS A FACTOR IN UROPEPSIN EXCRETION

Asher[3] considers stress of any kind, emotional or physical, to be the most important factor in the excretion of uropepsin. Studies of the general adaptation syndrome as related to stress point toward pituitary and adrenal control of this reaction, and investigation has been stimulated concerning the effect of steroids on uropepsin excretion.

High outputs of uropepsin are found in patients with Cushings disease,[24,43] hyperpituitarism,[43] and in those exposed to acute and chronic stress of a systemic nature such as surgery, myocardial infarction, burns, fractures and severe pain.[24] Patients who receive therapy with ACTH,[24,33] cortisone,[24,33] and testosterone[6]

also have increased excretion. Surgical vagotomy did not prevent the effects of steroids in guinea pigs, demonstrating that the stomach can respond to stress even if the vagi are absent.[33]

Low levels of uropepsin were found in Addisons disease.[24]

As a measure of exposure to stress, in the patient without gastrointestinal disease, the uropepsin determination gives similar results as the eosinophil count, 17 keto-steroid excretion levels and blood pressure determination.[3]

GASTROINTESTINAL DISEASE

The value of the uropepsin determination as a diagnostic aid in gastrointestinal disease has been investigated extensively since Farnsworth et al[19] made the initial study of its application to clinical medicine. The values obtained and the diagnostic accuracy of the test have varied from one investigator to another. The daily variation of individuals and the range of values even in normal patients makes the test difficult to apply in the individual case, although trends are established which are statistically significant in large series of cases.

Peptic Ulcer and Gastric Neoplasms - Patients with

duodenal ulcers excrete increased amounts of uropepsin[6,7,18,24,25,32,40,42,43,44] but only recently has it been emphasezed that uropepsin levels vary with the cyclic course of the disease, with high levels during the active phases, and normal levels during remissions.[44] The length of history of the ulcer and sex are considered unimportant, although values in males tended to remain above normal in remission more commonly than in females which returned to normal more consistently.[44] One author reports that in patients with a five-year history or longer, uropepsin values tend to increase.[29]

One study reports that although patients with duodenal ulcer have elevated HCl and gastric pepsin secretion, their uropepsin excretion is not significantly elevated. Vagotomy was reported in this study to lower gastric acidity, HCl and uropepsin levels.[42] Other authors report that vagotomy does not alter uropepsin excretion.[33,48]

The majority of patients with gastric ulcers excrete uropepsin at levels that fall within the range of normal.[15,21,44] Some authors have reported series in which the mean value for patients with gastric ulcers exceeded that of controls.[25,32] However, Sircus, in evaluating previous studies feels that they did not allow for coexisting duodenal and gastric ulcers. In his

series, 20% of patients with gastric ulcers had coexisting duodenal ulcers and 10% of those with duodenal ulcers had coexisting gastric ulcers. In the simple gastric ulcers in the series, however, uropepsin values were not increased significantly.[44] Lesions of the lesser curvature produce normal values while those beyond the lesser curvature produce increased values according to another series.[29]

It is generally agreed that patients with neoplasms of the stomach have reduced levels of uropepsin,[6,15,18,24,25] However, as mentioned before, the range of normals, and the range of values for ulcer patients overlaps the neoplasm group also, and in spite of statistically significant trends within any series, the results of determinations in individual cases cannot be used in establishing a definitive diagnosis without other confirmatory evidence of disease.

Uropepsin determinations may prove to be valuable in cases of hematemesis where barium studies cannot be made because of the patient's condition. In one series of nineteen cases of hematemesis, 12 cases with elevated uropepsin included 10 duodenal ulcers, 1 gastric ulcer and 1 aspirin gastritis while 7 cases with normal or low values included 3 peptic ulcers (site not mentioned), 1 chronic gastritis, 2 cases of hemorrhagic diathesis, and one case of esophageal varices.[44]

Other Gastrointestinal Diseases - Uropepsin excretion is increased in hypertrophic gastritis[15] and in patients with stomal ulcers.[32] Values are generally decreased in gastrectomy,[5,15,24,32] pernicious anemia,[1,24,32,34] atrophic gastritis,[5,6,15] and in hypochromic anemia without free acid.[1] Normal values are found in patients with steatorrhea,[1] gastroenterostomy,[15] and gastric complaints in the absence of a detectable lesion.[40]

ORIGINAL INVESTIGATION

The concept of exocrine-endocrine partition of pepsinogen with eventual excretion of the endocrine portion as uropepsin has been generally accepted. Most investigators feel, as mentioned previously, that the exocrine pepsinogen is either not absorbed or is rapidly altered by HCl to pepsin which is felt to be unabsorbable. This was shown by feeding crystalline pepsin and injecting pepsin intravenously, both techniques producing no increase in uropepsin excretion in humans and dogs.[36]

Because only crystalline pepsin had been given orally to study the possibility of pepsin absorption from the gastrointestinal tract, the effect of concentrated human gastric juice administered orally to patients with no intrinsic source of uropepsin was speculated upon. The presence of even a minute amount of a gastric enzyme,

normally absorbable by the gastro intentinal tract and absent in patients with pernicious anemia might in some way elucidate mechanisms of vitamin B_{12} absorption in the presence of normal gastric juice, even when fed to patients with pernicious anemia.

The technique of studying this problem would be to perform uropepsin determinations daily until a control level had been established. An ideal situation would be to discover a subject with no uropepsin excretion. The effect of administering human gastric juice orally upon uropepsin levels would then be determined. An increase in excretion, or excretion in a subject with no previous uropepsin excretion would demonstrate the absorption of a chemically active material by the gastrointestinal tract.

Before this could be done, however, several factors in the experiment were evaluated. These were the technique itself, the daily variation in excretion from day to day, and the gastric juice which was to be used.

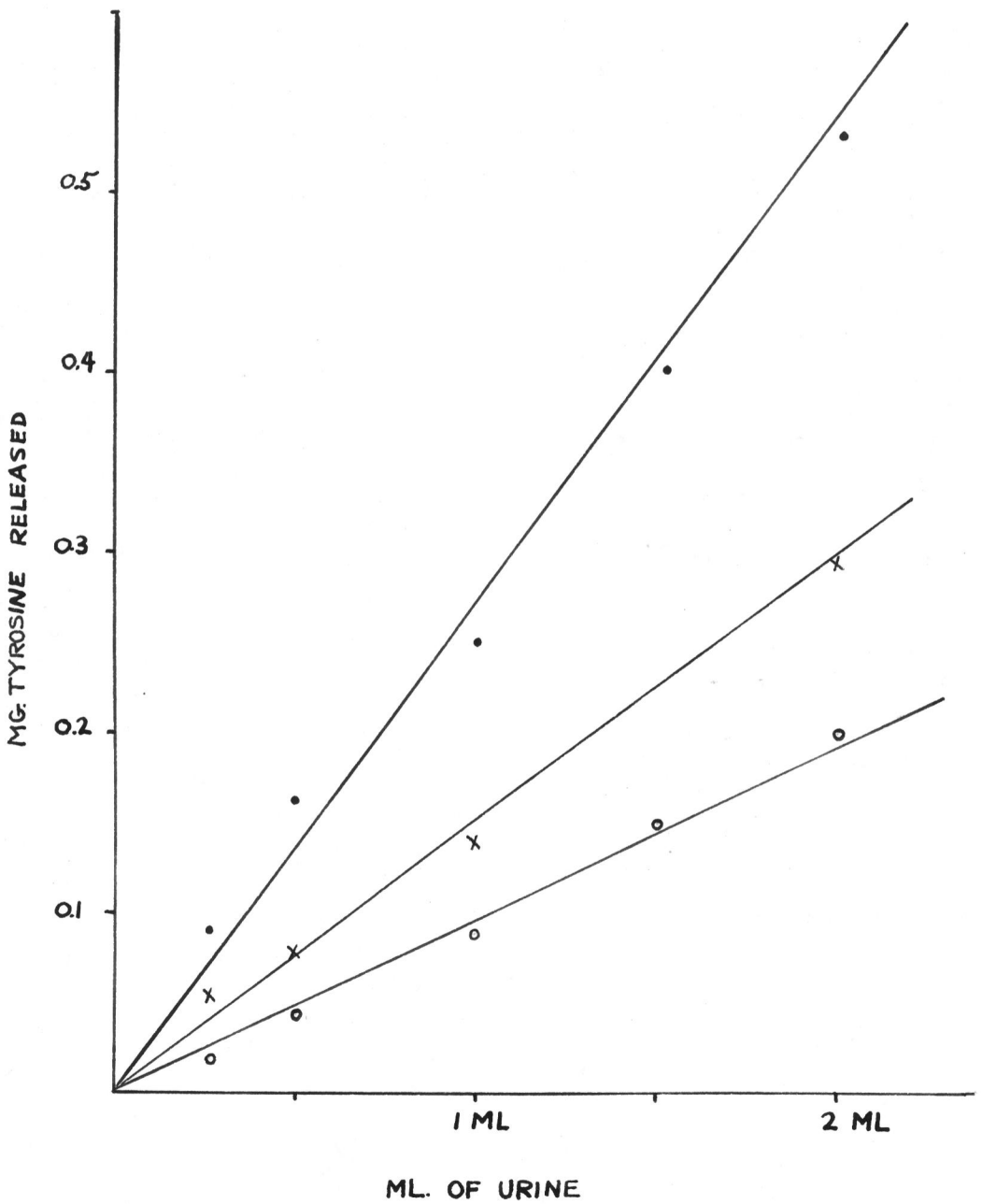
The method of Anson and Mirsky as described previously was used for all determinations. Dialysis of urine, as recommended by Duffin and Kowalewski[17] to reduce interference by urinary chromogenic materials (phenolic compounds) was not included, because another report[9] pointed out that dialysis was unnecessary because of inconsistency in the experimental results. The

dilution of the urine which occurs in dialysis is probably variable from sample to sample, making calculations unreliable.

The reliability of the technique was tested by performing multiple determinations on different aliquots of urine from the same twenty-four-hour sample from normal subjects. When 1 ml of acidified urine from different aliquots of the same urine was assayed for uropepsin activity, there was no significant difference between the results obtained. However, when samples of varied volumes were incubated (volumes ranging from .25 ml - 2.0 ml) the results were slightly scattered along a linear distribution. This demonstrates that dilution is an important factor, and because values obtained by assaying 1 ml must be multiplied by the volume of the 24-hour output of urine, even small dilution errors become important. (figure 1)

Uropepsin determinations were performed in a series of normal persons without complaints referrable to the gastrointestinal tract and compared with a group of patients with pernicious anemia. For ease of handling mathematically and graphically, 1 peptic unit was defined as that amount of activity which released 1 mg of "tyrosine-like" substances from acidified hemoglobin during a thirty minute incubation period. The following results

FIGURE 1



were obtained:

Patients with Pernicious Anemia

Subject Number	24 Hr. Vol.	Units / ml	Units / hr
1	370 ml	.03	.45
2	1329 ml	.03	1.66
3	1985 ml	.01	.82
4	540 ml	.03	.75
5	1360 ml	.00	.0
6	1010 ml	.02	.84
6	1120 ml	.0	.0
7	675 ml	.0	.0
7	668 ml	.0	.0
7	645 ml	.0	.0
8	1580 ml	.0	.0

Total 4.52 units per hour in eleven subjects. The mean value equals .41 units $\pm$ .53/hour.

Normal Subjects (males)

Subject Number	24 Hr. Vol.	Units / ml	Units / hr
1	775 ml	.09	2.9
2	615 ml	.10	2.6
2	720 ml	.21	6.3
3	1240 ml	.04	2.1
4	1790 ml	.03	6.7
4	1555 ml	.04	2.6
4	1530 ml	.04	2.5
4	1545 ml	.03	1.9
5	1420 ml	.12	7.1
6	1705 ml	.18	8.6
7	580 ml	.17	4.1
8	560 ml	.16	3.7
9	820 ml	.10	3.4
10	810 ml	.22	7.4
11	1705 ml	.13	9.0
12	895 ml	.06	2.2
12	1000 ml	.11	4.5
12	1270 ml	.07	4.1
12	1240 ml	.11	5.7
12	1440 ml	.10	6.0

Total 93.4 units per hour in twenty determinations. The mean falue equals 4.7 ± 2.2 units / hour. Conversion to the units of Gray and Hirschowitz produces mean normal values of 2808 units/24 hrs and 261 units / hr respectively. These values compare favorably with the normal mean of 2300 units / 24 hrs established by Gray et al,[23] and the normal mean of 244 units / hr established in a series of makes by Hirschowitz.[29]

The graphic comparison of the series presented above appears in Fig. 2.

Uropepsin determinations were also performed on two individuals for four successive days in case. The results are presented graphically in Figures 3 and 4 with the 24-hour urine volumes.

One Patient among the pernicious anemia series was found to excrete no uropepsin even with repeated assays, and he was felt to be an ideal subject for studying the effect of oral human gastric juice. Determinations were performed to establish a basal level during which period no uropepsin was excreted for four consecutive days. On the fifth day the patient received 1000 ml of reconstituted gastric juice, 250 ml before each meal and 250 before retiring.

This was gastric juice which had been previously collected from a normal subject after histamine injection,

FIGURE 2

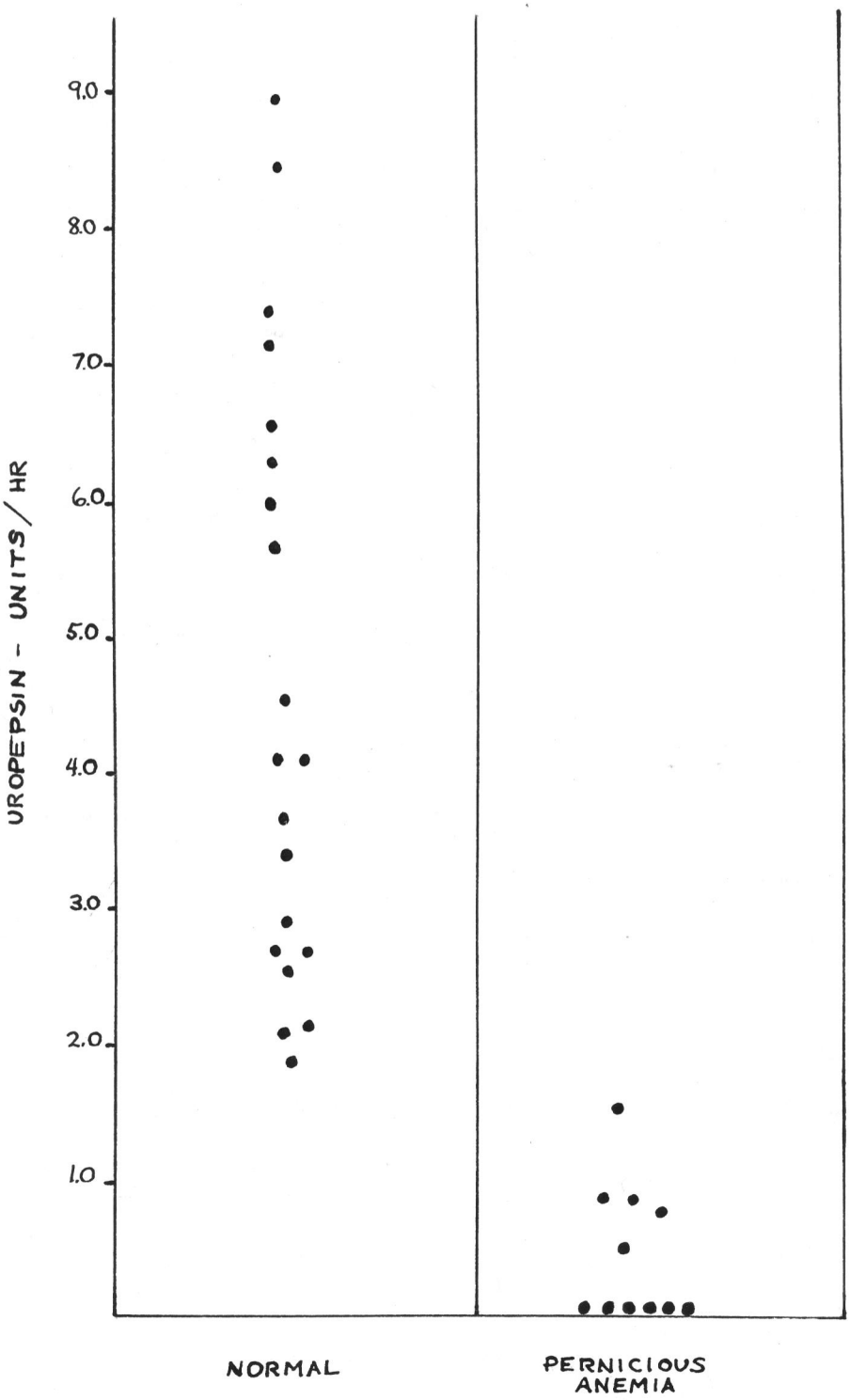

UROPEPSIN - UNITS / HR
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
NORMAL
PERNICIOUS ANEMIA

FIGURE 3

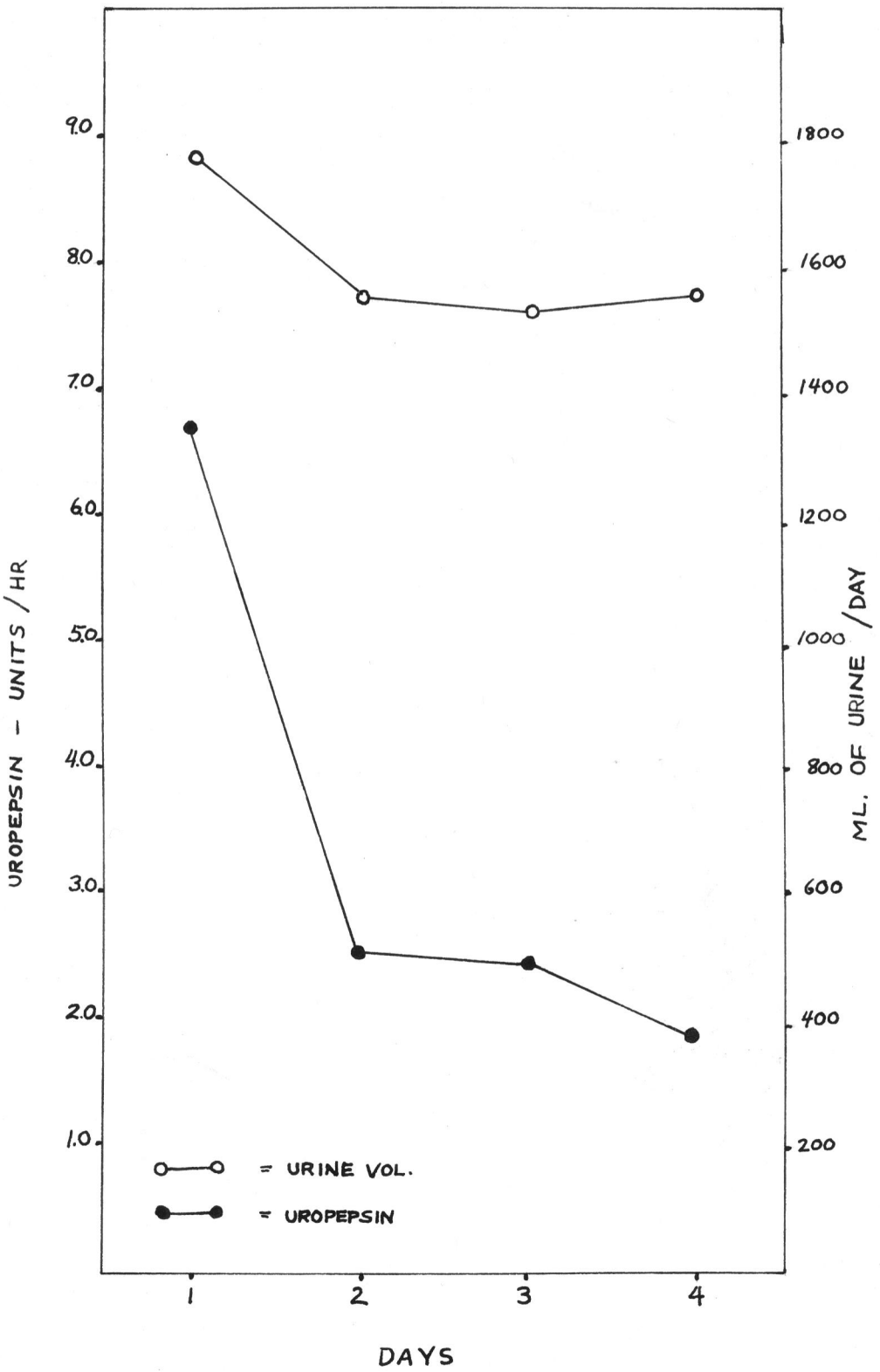
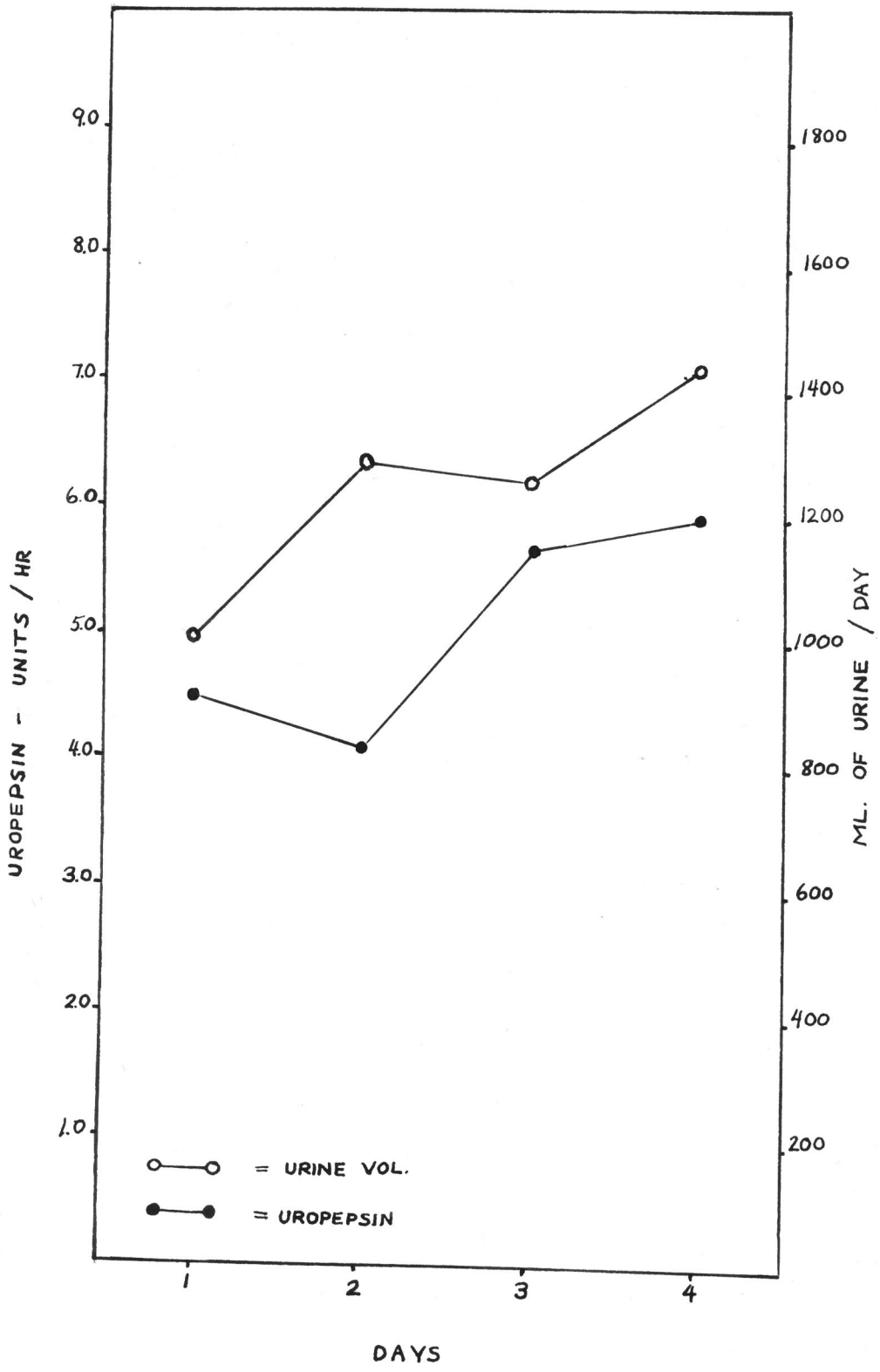

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
UROPEPSIN - UNITS / HR
1800
1600
1400
1200
1000
800
600
400
200
ML. OF URINE / DAY
= URINE VOL.
= UROPEPSIN
1
2
3
4
DAYS

FIGURE 4


9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
UROPEPSIN - UNITS / HR
1800
1600
1400
1200
1000
800
600
400
200
ML. OF URINE / DAY
= URINE VOL.
= UROPEPSIN
1
2
3
4
DAYS

neutralized with NaOH, dialyzed, lyophilized and frozen. It was reconstituted by thawing and adding distilled water to the desired volume. Peptic activity of the juice was determined by adjusting the pH of a 20 ml portion to 1.5, making the volume to 25 ml and assaying 1 ml by the same technique used for assaying urine. 1000 ml of this gastric juice contained 140 peptic units.

Uropepsin determinations performed on the urine excreted on the day of ingestion and the following day revealed still no excretion of uropepsin. This would probably indicate that no absorption in this subject had occurred. However, the gastric pepsin levels induced by this technique were not as high as might possibly be obtained with gastric juice containing greater proteolytic activity. Determinations on gastric juice from other normals revealed peptic activity ranging from .7 units/ml - 6.9 units/ml. The use of one of the more active juices would have been more revealing.

The normal patients studied excreted a mean of 112.8 peptic units/24 hrs. If, according to Janowitz and Hollander,[31] only 1 per cent of the pepsin produced is excreted in the urine, it would be more realistic to give 11,000 peptic units orally to obtain normal values (assuming that absorption occurs).

It would be interesting to administer gastric juice

from patients receiving steroids to patients who did not excrete uropepsin. By this method a more concentrated peptic activity could be produced.

SUMMARY

Uropepsin, an enzyme present in urine in normal subjects has been investigated extensively by many workers as a technique for diagnosis of diseases of the gastointestinal tract. Because the final excretion of the enzyme in the urine is affected by the gastric peptic cell mass, the proportion of pepsinogen which is secreted into the stomach as compared with that into the blood, the renal clearance of pepsinogen, the stress situation of the individual, and daily deviations, the assay has not been accepted as a basic technique in diagnosis. Technical details of the test itself do not at present make it available to the average general hospital.

As a research technique, the test is not often diagnostic in the individual subject, but becomes statistically significant in large groups of subjects.

Determinations on a small group of patients with pernicious anemia indicate that the presence of this disease might be ruled out by uropepsin determinations.

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APPROVED

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