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THESIS FOR THE DEGREE OF

DOCTOR OF MEDICINE

AT THE UNIVERSITY OF THE WITWATERSRAND.

A STUDY OF THE PHYSIOLOGY OF PEPSINOGEN IN THE HUMAN WITH SPECIAL REFERENCE TO ITS DISTURBANCE IN DISEASES OF THE UPPER GASTRO-INTESTINAL TRACT.

Presented by

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INTRODUCTION.

This study was originally undertaken to determine the clinical value of estimating pepsinogen in the urine in cases of peptic ulcer because recent publications had shown that uropepsin (urinary pepsinogen) excretion in ulcers differed from normal controls.

Briefly, the state of knowledge of pepsinogen when this study was undertaken was as follows: pepsinogen was discovered by Langley in 1881 and eventually isolated in 1938 by Herriot who described its physico-chemical properties. It was known to be formed in the stomach wall and mainly secreted into the stomach where it was irreversibly changed into pepsin in the presence of hydrochloric acid. None was reabsorbed and this pepsin was destroyed in the alkaline small intestine. Some pepsinogen diffused directly into the blood stream from the peptic cells and as far as could be ascertained was lost, till it appeared in the urine - a fact discovered by Brücke in 1861. Its transport to and the mechanism of its excretion by the kidney were the subject of hypothetical discussion only. In disease urinary pepsinogen had been studied for some time and the final conclusions were that it was increased in ulcers, especially duodenal ulcers and decreased or absent in pernicious anaemia.

It was apparent early in this study that investigation of urinary pepsinogen alone would be of little value in advancing the knowledge of the normal and abnormal physiology of pepsinogen in the human. The investigation was then extended to study the pathological disturbances of urinary pepsinogen more closely, and if possible to determine what happened to pepsinogen between the stomach and the urine. This latter project became possible after the development of a technique not previously described for determining blood pepsinogen and it was found that pepsinogen diffuses from the peptic cells into the blood, and circulates as free pepsinogen in the plasma. From the plasma it is freely diffused through the body and filtered through the glomerular membrane and then reabsorbed in the tubules of the kidney to the extent of 65 - 95%.

This new concept of pepsinogen excretion by the kidney calls for a re-orientation of the conclusions previously held of the significance of the urinary pepsinogen in disease.

In this report, an attempt is made to present the whole cycle of pepsinogen metabolsim from its formation in the stomach to its appearance in the urine, with normal and abnormal variations, as a unified concept. While in places comments or conclusions may appear dogmatic, it is realized that this study has produced more questions than answers. It is hoped, however, that a small contribution will have been effected by this work towards a better understanding of the direction of further advances in the knowledge of the aetiology and pathological behaviour of peptic ulcers.

ACKNOWLEDGMENTS.

This study was undertaken on the advice of Dr. F. Avery Jones and has been pursued under his direction and sustained by his constant advice and encouragement, without which it would have foundered; I find it hard to express adequately my deep gratitude to him.

My sincere thanks go to the members of the Department of Gastro-enterology, especially Dr. Richard Doll, for their assistance and co-operation and to Mr. R.A.Heady for his advice and assistance with the statistical calculations. Dr. George Discombe and his staff, especially Mr. Raymond Jones, of the Biochemistry Department, were extremely helpful on the technical side.

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CHAPTER I

HISTORICAL INTRODUCTION: A SHORT HISTORY OF DIGESTION

"In the alimentary canal many wonderful processes take place" - Cicero.

The origins of man's knowledge of food and speculations on digestion are lost in the mists of pre-history. Hippocrates (460 - 377 B.C.) in De Prisca Medicina states: "It was the necessity which men in the first stages of society must have felt of ascertaining the properties of vegetable productions as articles of food that gave rise to the science of Dietetics; and the discovery having been made that the same system of regimen does not apply in a disordered as in a healthy condition of the body, men felt compelled to study what changes of the aliment are proper in disease and it was the accumulation of facts bearing on this subject which gave rise to the Art of Medicine." (translated by Francis Adams - 1849).

The first known views of man on health and disease, the Ebers papyrus, dating back to 1550 B.C., show us that the Ancient Egyptians considered health an abstract idea and disease a concrete thing. They believed that all disease originated from food and followed this precept by regularly taking an emetic and a purgative three times a month. They regarded hunger and thirst as poisonous substances which forced themselves into the body, requiring food and drink to

be subdued and neutralized to save the body from destruction.

Since that time, the history of digestion is in part the history of medicine. From 1550 B.C. at least, until the doctors of Greece began to dominate medical thought from the 5th century B.C., the views of the Egyptians, the Hindus, the Hebrews and the Chinese were rather similar and consisted of one form of demonology or another.

The Hindu views on digestion are known from the epic Hindu poems and are indirectly related to the demonological views of the Egyptians. These writers recognized three principal humours which resided in the body and directed the natural digestive processes.

These humours were:

Vata or wind situated between the feet and the navel;

Pitta or bile between the navel and the heart;

Kafa or phlegm between the heart and the top of the head.

Kafa was dominant when the food was in the mouth, Pitta during the course of digestion and Vata after the food in the stomach was fully digested, and the condition of the bowels depended on the undue dominance of one of these - Vata caused constipation, Pitta looseness of the bowels, and Kafa a normal action.

These writers also describe a Pachaka or fire of digestion situated between the stomach and the small intestines which

assisted digestion and imparted heat to the body, and separated the nourishing portions of the food from the dejecta. The external elements which brought about changes of food occurring in contact with the Pachaka or digestive fire, were five in number - ether, fire, air, earth and water. The dietetic rules of the Hindus were extremely minute and they generally forbade meat.

The Hebrew medicine of the same period was an off-shoot of the early dynastic Egyptian but in addition the dietetic rules were far stricter and survive in an almost unchanged form today. The laws relating to Kashris or Kosher food prohibit the mixing of meat and milk and forbid the use of meat from pigs, from the hindquarter of animals unless specially dealt with and from animals with uncloven hoofs; they forbid the eating of fish without scales. While some of these laws have their basis in primitive ideas based on the supposed incompatability of milk and meat etc., many were based on sound public health reasons, e.g. the forbidding of pigs' meat was based on the frequent disease (measles) encountered in pigs.

The Chinese physiology regarded the bile as the seat of courage, the spleen of reason and with the assistance of the heart of ideas; the liver as the granary of the body and the stomach as the abode of the mind.

From an early period, the stomach was generally recognized as the chief organ of digestion and much attention was paid to

it by the earliest authors on medical subjects.

The early Greeks of the pre-Dogmatic or pre-Hippocratic era believed that the food putrefied or was cooked in the stomach. Anaxagoras of Clazomedae (500 - 428 B.C.) believed that the body made use of the products of digestion by appropriating to itself those portions most akin to it.

Hippocrates (460 - 377 B.C.), who was the founder of the Rational school, believed that digestion was a process of coction in which heat brought out the necessary changes in the food.

Hippocrates separated the active agents of the body into four principal humours - blood, phlegm, black bile and yellow bile, as other Greeks had done before him, and Heat, Cold, Dryness and Moisture were their corresponding properties. Heat, due to the blood, caused digestion of the food, the liver formed blood and bile and assisted the process of coction in the stomach by increasing the heat. Hippocrates founded the science of dietetics and both in the discourse on ancient medicine and in his aphorisms, detailed references to diet in disease abound. These aphorisms were the mainstay of dietary regimes well into the 19th century (Adams 1849).

Following Hippocrates, who divorced philosophy from medicine, and based his remarks on observations, partly empirically, came a decline in the form of a re-marriage of philosophy and medicine, based on Plato's (427 - 347 B.C.) fanciful and speculative scheme of physiology and pathology, which led to the founding of the Dogmatic school. Praxagoras of Cos (circa 335 B.C.) one of that school

who recorded the first known case of bulimia, and described the treatment for intestinal obstruction, regarded digestion as the outcome of putrefactive processes.

Aristotle (384 - 321 B.C.), like Hippocrates, regarded digestion as a process of coction in the stomach, from which the resulting chyle went to the heart, and he regarded the blood flowing through the vessels as the medium by which the nutritive material for the growth and warming of the body was conveyed.

The early Roman era that followed believed that all the organs of the body were supervised by special divinities who could be appealed to if anything went wrong.

The most famous of all Roman physicians, Claudius Galen of Pergamus (131 - 201 A.D.) followed about 500 years after Hippocrates and was destined to dominate the medical world for more than a thousand years. Galen believed that digestion of the food was brought about by coction in the stomach; the results of digestion went to the liver where they were turned into blood (this belief persisted to the 17th century). The blood upon reaching the heart was driven to all parts of the body and was almost entirely used up in transit in nutrition of these parts.

Little was added during the Dark Ages to knowledge in general and to Medicine in particular, though the Arabic schools of

science did produce some famous doctors from about the 9th to the 13th centuries, none advanced the knowledge of digestion in any way.

Medical science lagged behind in the dawn of the Renaissance. Bombastus Paracelsus (1493 - 1541)(Theophrastus von Hohenheim) believed that digestion was carried out in the body by a presiding force called the "archeus" which separated the nutritious from the poisonous ingredients of the food and helped absorb the nutriment. This it did solely by means of heat. These ideas are reminiscent of the ancient demonology. However, let us not linger on the theories of Paracelsus, since his reputation far exceeded his merits.

The foundations of modern knowledge of digestion were laid by Johan van Helmont (1577 - 1644). His interest was aroused as a young boy when he fed sparrows and let them get hold of his tongue; he noticed the intense acidity in the birds' throats. Later, he saw some sulphurous substance reduce his glove partly to fluid and not unnaturally he believed that the digesting agent in the stomach was acid. He also believed that this acid was peculiar to each species of animal.

In denying Paracelsus' theory of heat as the digestive agent he argues that "hunger and strong and molesting digestion should be accompanied by excessive heat. This is not the case .....When food is brought into contact with a warm surface there is no trace of digestion and it putrefies". (Pagel 1944). He states further that

"warmth acts in a uniform way, the specific vital ferments act according to the exigencies of the species and living being in question". The vital faculty of transforming food ("vera atque formaliter") Van Helmont called "Ferment" ("Eamque fermentorum nomine designavi") and enlarging upon this states: "A digestive ferment, therefore, has the essential power by reason of its vital acidity of causing transmutations.

In binding up his theories of digestion as fermentation with the doctrine of material life, Van Helmont may today appear ludicrous but he must nevertheless be given credit for being one of the first to break through the artificial fabric of medical science erected by Galen and the succeeding thousand years. Sylvius, Descartes and Willis (17the century) belonged to this new school and identified digestion with fermentation.

Nehemiah Grew (1626 - 1711) noticed the glands in the stomach and in a lecture said "By the joynt assistance of the glandulous and nervous membranes, the business of chylification seems to be performed. The mucous excrement provided by the former, as an animal corrosive, preparing, and the excrement of the nerve by the latter, as an animal ferment, perfecting the work". (Gillespie,1898).

Following the inconclusive attempts of the chemical school to provide an explanation of digestion came those of the Iatro-mathematical school of physiologists who attempted to show that digestion (and other physiological processes) could be explained on a purely mechanical basis. Redi(1626 - 1698), one of the leaders of this

school, studied the mechanism of the gizzard of birds very carefully and this suggested to him a similar action by the human stomach. Some were prepared to go to preposterous lengths and Dr. Pitcairn, by simile with pressure exerted by the thumb, estimated the grinding force of the human stomach at about 5 tons and this he believed dispensed with the "assistance of a Daemon or a Stygian Liquor".

The Eclectic school originating with Hermann Boerhaave (1668 - 1738) believed in the mechanical theories of digestion and added little to what was believed.

The system of vitalism introduced by Borden of Montpelier (1722 - 1776) and supported by Barthez (1734 - 1806) believed that a "vital principle" was the cause of all the phenomena of life including digestion. This was opposed to the conception of force and matter.

Edward Rigby (1747 - 1821) of the Phlogistic school, held that the stomach was the seat of "phlogistication" where free heat was produced.

The 18th century produced firstly the observations of Reaumur (1683 - 1757) in about 1710 who obtained pure gastric juice and found it acid and aseptic.

An observation widely ascribed to Spallanzani in 1783, viz. the fact that gastric juice could digest outside the body, was actually made 5 years before by Stevens and merely corroborated by Spallanzani.

The 19th century produced a great advance in the knowledge of digestion and this can best be shown in a chronological symopsis

(taken from Gillespie 1898).

1814 - Trevisanus finds a sulphocytanide in saliva.

1824 - Prout, Tiedemann and Gmelin independently discover the presence of free hydrochloric acid in gastric secretion.

1825 - 1833 - William Beaumont's observations through a gastric fistula.

1829 - Arnot proposes a stomach syphon.

1831 - Leuchs discovers the diastatic action of saliva.

1834 - Eberle prepares an artificial gastric juice.

1836 - Schwann postulates the existence of pepsin; makes first biliary fistula.

1839 - Wasmann isolates pepsin.

1842 - Bassow and Blondlot make artificial gastric fistula in the dog.

1846 - Claude Bernard: Theory of hepatic glycogenesis, fat emulsifying ferment in pancreas, etc.

1847 - Dubrunfaut isolates maltose.

1851 - Heintz finds the milk-curdling ferment.

1851 - Lehmann finds lactic acid in gastric content.

1852 - Bidder and Schmidt prove conclusively that hydrochloric acid is secreted by the gastric glands.

1854 - Busch finds no secretion in the small intestine in absence of stimuli.

1854 - Ebstein and Grützner postulate the existence of pepsinogen.

1859 - Meissner investigates the products of the gastric digestion of proteids.

1861 - Brücke discovers pepsinogen in the urine.

1864 - Städeler investigates the bile pigments. Thiry analyzes juice of small intestine by means of an artificial fistula.

1871 - Paschutin discovers sugar-inverting ferment in small intestine. Eichhorst shows that ferments are absent in mucous membrane of large intestine.

1873 - Liversidge demonstrates the presence of a zymogen of the diastatic enzyme in the pancreas.

1875 - Heidenhain discovers proteolytic zymogen in the pancreas etc. Darwin describes digestion of proteids by plants

1878 - Kühne investigates the products of tryptic digestion of proteids. Richet finds that hydrochloric acid in the stomach contents is often combined with organic bodies.

1881 - Vella modifies Thiry's fistula.

1881 - Langley discovers pepsinogen in the gastric mucosa.

1882 et seq. - Langley investigates the influence of reaction on ferments.

The 20th century has witnessed tremendous advances in all branches of science and the science of medicine today is far removed from the art of Hippocrates. The study of digestion has been graced with the title of gastro-enterology flanked by the science of nutrition and supported by special branches of enzymology, chemistry and histology.

It would be difficult to summarize the history of digestion of the last half-century except by saying that we are constantly learning how little we have learnt and more scientifically knowing how little we know. The history of digestion is one of the discovery and application of new techniques - radio-isotopes are no more or less important than the Rehfuss tube; the gastroscope and

the x-ray than the haemoglobin method for measuring peptic digestion or the potentiometer for measuring pH, and further advances of knowledge must wait for developments in the field of technology.

THE HISTORY OF PEPSINOGEN

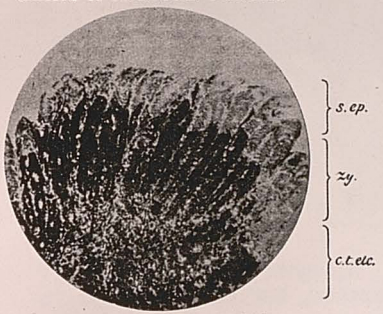
The history of pepsinogen is part of the latter and more scientific knowledge of digestion. As such it followed knowledge of peptic digestion by ferments, and the discovery of pepsin. Of necessity, the story of pepsinogen is intertwined with that of pepsin, since no advance in the knowledge of pepsinogen was possible, up to a point, without a sound method for the assay of pepsin.

In 1834 Schwann postulated the existence of pepsin and it was not until 1839 that Wasmann discovered pepsin, 200 years after van Helmont had called the vital faculty of transforming food a ferment. (Eamque fermentorum nomine designavi).

Following the discovery of pepsin the site and method of its production was the subject of much argument until Langley (1881) conclusively proved both and concluded that "The fresh gastric glands contain no pepsin; they do, however, contain a large quantity of pepsinogen; consequently the granules of the chief cells consist wholly or in part of pepsinogen."

In 1898 Gillespie published the first photograph of pepsinogen granules in the gastric mucosa, which is reproduced below.

MICRO-PHOTOGRAPH OF THE GASTRIC MUCOUS MEMBRANE OF THE *SALMO SALAR*, SHOWING AGGREGATIONS OF PEPSINOGEN GRANULES.



(Photograph by Dr. Lovell Gulland.)

s.ep. Surface epithelium.
zy. Zymogen granules in the cells of the glands.
c.t., etc. Connective tissue, blood-vessels, etc.

The photograph was taken from the mucous membrane of a kelt when the glands were growing again on descent from the spawning bed to the sea.

Fig. 1. FIRST PUBLISHED PHOTOGRAPH OF PEPSINOGEN (Gillespie 1898)

It was many years between the discovery of pepsinogen in 1881 and its isolation in the crystalline form by Herriot in 1938. Herriot studied its physico-chemical properties fully and his results confirmed the accuracy of much of Langley's observations on the inactivation of pepsinogen. Much of the work done on pepsinogen in the period between its discovery and its isolation suffered from the lack of a suitable method for peptic assay.

The knowledge of how pepsinogen behaves in the body has been rather painfully acquired during the 92 years since Brücke (1861) discovered it in the urine. The history of this aspect has been fully reviewed in Chapter IV and will not be discussed here.

Little remains to be said of the history of this substance, but much remains to be discovered about this ferment that we have for so long taken for granted.

CHAPTER II

PHYSICO-CHEMICAL PROPERTIES OF PEPSINOGEN

Since Langley (1881) identified a substance in gastric mucosa which in a slightly alkaline extract was not pepsin but which changed to pepsin upon acidification, and which he identified as pepsinogen, attempts have been made to isolate this substance. Holter and Northrop (1935) first prepared a partially pure substance by fractional salt precipitation of extract of swine mucosa which was about half as active as pepsin. Herriot (1938) went further and was the first to prepare pure pepsinogen from swine gastric mucosa and was then able to study its various physico-chemical properties.

Since the work of Herriot (1938) has been little changed since then, the following pages dealing with the subject of the chemistry of pepsinogen are largely taken from his fine paper.

Method of Isolation:

Of the total content of about 1 gram of pepsinogen in the average swine stomach, about 90% is found in the fundus. The minced fundus mucosae were extracted with a bicarbonate ammonium

sulphate solution, followed by precipitation of the soluble pepsinogen at a higher concentration of ammonium sulphate. By this part of the process alone, 80% of the total pepsinogen was recovered. This preparation was 50 times as potent as the original minced fundus and half as pure as the fine crystalline product (a stage reached, at best, by Holter and Northrop (1935)). No further improvements could be made by further fractional salt precipitation, and this product contained a considerable admixture of carbohydrate and of protein impurities. When this pepsinogen solution was mixed at pH 6.0 with a washed copper-hydroxide solution, practically all the protein and carbohydrate were bound to the copper precipitate. When extracted with a phosphate buffer of pH 6.8, 70% of the pepsinogen went into solution with little of the impurities. By repeating this treatment with copper hydroxide and phosphate buffer a pepsinogen preparation was obtained which contained practically no carbohydrate and less than 5% of protein impurities. No further improvement in quality was obtained by Herriot (1938) with further procedures.

Crystallization of the pepsinogen after two treatments with the copper reagent was carried out by dissolving an 0.7 saturated ammonium sulphate filter cake in 9 volumes of 0.4 saturated ammonium sulphate - M/10 pH 6.3 phosphate buffer and stirring the solution slowly at 10° C.

"In 6 - 24 hours the beaker was a swirling mass of very thin long needles of pepsinogen" (Herriot 1938) (see plate). This method has proved reproducible and accurate.

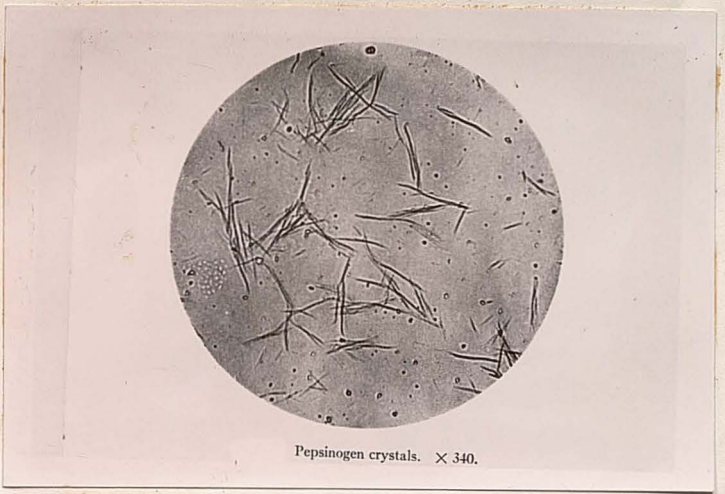


Fig. 2. FIRST PUBLISHED PHOTOGRAPH OF CRYSTALLINE PEPSINOGEN (Northrop, Kunitz and Herriot, 1948).

Tests of Purity:

Having obtained a crystalline product, Herriot had to determine whether it was a pure substance.

(a) The first test applied was a solubility experiment on the basis of the phase rule, which states that for a given solvent the solubility of a pure substance is independent of the quantity of solid phase, i.e. the amount dissolved will not depend on the amount of solid substance after saturation.

The solubility of pepsinogen in 0.55 saturated ammonium sulphate buffer was found to be constant over a 20-fold variation in the quantity of solid pepsinogen. The dissolved amount was

checked in 2 ways - viz. estimation of dissolved protein nitrogen, and the amount of pepsin produced upon acidification (tested both by haemoglobin and rennet methods).

(b) Other evidence pertaining to the purity of the pepsinogen was of a negative character and was the result of trying to separate the hypothetically pure pepsinogen from any impurity. The purity was tested by the amount of pepsin produced upon acidification (potential activity). This was used because the fundamental and characteristic property of pepsinogen is its quantitative conversion to pepsin, combined with the fact that the latter can be measured with a precision of nearly 5%. The activity of the pepsinogen is determined per mgm. of protein nitrogen in the preparation. This activity was determined before and after fractionation and fractional recrystallization; this was also done after partial heat and alkali denaturation and after partial reversal of completely denatured pepsinogen by heat and alkali.

Herriot concluded that pepsinogen as isolated was a pure protein which withstood the tests for protein purity within the limitation of the method, that is, about 5%

Properties of Pepsinogen

These are summarized in the table taken from Herriot (1938) where they are compared with pepsin.

COMPARISON OF PROPERTIES OF SWINE PEPSIN AND PEPSINOGEN(Herriot 1938)

PROPERTY	PEPSIN	PEPSINOGEN
Crystalline form	Hexagon bipyramids	Needles.
Catalytic Activity	+	-
Specific activity or specific potential activity P.U. mgm. P.N.	0.26	0.22
$[\alpha]_D^{25°C}$ pH 5.6 (optical rotation) per gm. of dry protein	- 72°(-71°)	-82° (-81°)
Isoelectric point	pH 2.7	pH 3.8
Molecular weight (osmotic pressure)	38,000± 3,000	42,000 ± 3,000
NH_2-N% of total nitrogen	1.4	4.0
Reversibility of denaturation	1.0%	80 - 100%
Hydration or Molecular shape.	Hydrated or non-spherical	Non-hydrated or spherical
pH stability	pH 2.0 - 6.0	pH 6.0 - 9.0

pH of alkaline inactivation at 25°C	7.0	9.4
Tyrosine-tryptophane content	At pH 11 - 9.3% At pH 8.0 - 5.5%	At pH 11 - 8.6% At pH 8.0 - 5.2%
U.V.L.Absorption spectrum	Same	Same

Elementary analyses.
% of dry protein

C	51.7	52.8
H	6.8	6.9
N.Dumas	14.7	14.2
N.Kjeldahl	14.0	13.9
S	0.45	0.40
P	0.09	0.08
Cl.	0	0

It will be seen from the table that those properties which depend on the whole molecule are much the same in the two proteins, whereas those which depend on a functional part of either or on a particular function are in a number of instances different. Thus the elementary analyses, molecular weights, absorption spectra, and optical rotation are qualitatively the same in both proteins, whereas enzymatic activity, pH stability, isoelectric point, amino-nitrogen, titration curves, reversibility of heat and alkaline denaturation and antigenicity (Seastone and Herriot 1957) are quite different in the two proteins.

One of the most important differences and one on which much of the identification of pepsinogen and pepsin in body fluids is based, is on the difference of denaturation between pepsin and pepsinogen,

denaturation being one of the characteristics of protein. Alkali denaturation of pepsin occurs at appreciable rates in solutions more alkaline than pH 6.0. The reaction follows a simple monomolecular course with an increase of the rate of denaturation with an increase of pH. In concentrated solutions the denaturation is not reversible. Heat denaturation also follows a monomolecular course (Northrop 1930) and is not readily reversible.

Pepsinogen, on the other hand, is also denatured by heat and in solutions more alkaline than pH 8.5 but this denaturation is completely reversible. Complete denaturation may be demonstrated by the inability to redissolve the protein in salt solutions in which the native form was soluble and by the fact that it cannot be converted to pepsin when it is acidified, and reversibility may be shown by a complete return to its original physical properties. This denaturation resembles denaturation of trypsin by heat (Anson M.L. and Mirsky A.E. (1934a) or of haemoglobin by salicylate (Anson M.L. and Mirsky A.E.)1934b).

This denaturation is a true equilibrium reaction as can be shown by the fact that at any temperature or pH in heat or alkali denaturation, the quantity denatured is the same whether that temperature or pH is approached from the higher or the lower value, i.e. whether one starts with the denatured or the native form.

Upon long exposure of pepsinogen to the denaturing agents, the reversibly denatured material is slowly changed to an

irreversibly denatured form. The rate of this change is increased by an increase in temperature or pH and in the case of the heat denaturation this reaction follows the monomolecular form. At about 50°C. at pH 7.0 and with 1 mg. protein N. per ml., the denaturation of pepsinogen is near the equilibrium midpoint, i.e. half denatured and half active.

To put the above observations in other words:

(a) Reversible heat inactivation: Neutral, salt-free pepsinogen solutions may be heated to boiling and then cooled without loss of potential activity. If salt is added to the solution the denatured form of the protein is precipitated, and by this means it can be shown that at 50°C. pepsinogen is practically completely in the native form and at 70°C. preactically completdy denatured, indicating a very high heat of reaction.

(b) Reversible alkali inactivation: At pH 7.0 pepsinogen is entirely in the native form, at pH 8.5 to 10.5 it is denatured, but this denaturation is for a time reversible. If the logarithm of the ratio of native to denatured pepsinogen at 25°C. is plotted against pH, a straight line with a slope of 2 is obtained. This shows that the following reaction expresses this equilibrium (Northrop, Kunitz and Herriot 1948).

$$\text{Native pepsinogen} + 2\ OH^- \rightleftharpoons \text{Denatured pepsinogen } (OH^-)_2$$

or

$$\log \frac{\text{Pepsinogen}}{(\text{Pepsinogen}(OH^-)_2} = -2 \log(OH^-) + \log K$$

These two properties of reversible heat and alkali denaturation are entirely different between pepsinogen and pepsin. Temperatures over 70°C. or pH over 7.0 instantly and irreversibly denature pepsin.

Titration curves.

Titration of pepsinogen or pepsin between pH 6.0 and 12.0 shows that pepsin (which on the alkaline side of pH 6.0 is denatured) combines with more alkali between pH 6.0 and 9.0 and pepsinogen between pH 9.0 and 12.0, each corresponding to the pH of maximal denaturation; though the total groups combining with alkali between pH 6.0 and 12.0 is the same in the two proteins (Northrop, Kunitz and Herriot 1948), confirming Steinhardt's(1937) suggestion that alkali inactivation of pepsin is the result of ionization of a series of groups. Below pH 6.0, the comparison is invalid because of conversion of pepsinogen to pepsin.

Ultraviolet absorption spectra.

Ultraviolet spectra show little difference between pepsin and pepsinogen, though one of the tyrosine absorption bands appears a little different in pepsinogen (Herriot 1938).

Immunological relation of pepsin and pepsinogen

Studies on pepsin are complicated by the fact that this protein is denatured in the blood stream (pH 7.0) almost immediately upon injection with corresponding antibodies to the

denatured and not the active pepsin and it is known that denatured proteins are not as distinct immunologically as the native forms. This might explain the sometimes non-specific results in that study. However, it was found by these authors (Seastone and Herriot 1937) that pepsin from a swine, cattle and guinea-pig reacted with serum from a rabbit immunized with swine pepsin, but pepsin from a rabbit chicken or shark did not. This shows that only partial species specificity exists.

Pepsin antisera that reacted with pepsin, reacted with pepsinogen from the same species but not with the blood proteins. The pepsin is therefore immunologically a foreign protein. On the other hand, antipepsinogen sera do not react with the corresponding species pepsin and the reaction between pepsin antisera and pepsinogen may be due to the unavoidable denaturation of pepsin when injected, whereas pepsinogen remains a native protein at the pH of blood.

Immunization sera made with pepsinogen do not react with same species serum proteins, nor do serum protein antisera react with either same species pepsin or pepsinogen.

In summary, therefore - pepsin and pepsinogen have two distinct specificities. They are immunologically distinct from each other, from normal serum proteins and generally from pepsin of other species, thus showing species specificity. This

specificity is similar to that of lens protein (Northrop, Kunitz and Herriot 1948).

Isoelectric point.

This was determined by Herriot (1938) by cataphoresis measurements of pepsinogen coated collodion particles suspended in m/10 acetate buffers of various pH values, and found to be 3.7. This was checked by finding the pH at which either the minimal amount of alcohol precipitated pepsinogen or the maximum precipitate was obtained with a constant amount of alcohol. This had to be done at 0°C. before any appreciable activation took place. The range of results varied from pH 3.6 to 4.3 with 3.9 as the most likely isoelectric point. That for pepsin is pH 2.7 (Northrop 1930).

Amino Nitrogen

The amino-nitrogen content of pepsinogen is much higher than that of pepsin (15 - 20%) corresponding to the 9 amino groups split off during activation and part of which (5 groups) is thought to form the dissociated inhibitor complex with pepsin between pH 5.0 and 6.0 (see below).

Molecular Weight.

The difference in molecular weight between pepsin and pepsinogen is accounted for by the amino groups split off during activation and as determined by Herriot (1938) are 38,000 ± 3,000 for pepsin and 42,000 ± 3,000 for pepsinogen. These are calculated from osmotic pressures and are probably no more than approximate.

Tyrosine-Tryptophane Content.

These were determined on the denatured forms of both pepsin and pepsinogen and found to be the same (Herriot 1938). Since the tyrosine can only be measured by the Folin-Ciocalteau phenol reagent at alkaline pH, it is not possible to determine the tyrosine content of pepsin in its native form. That of pepsinogen in its native form is lower than in its denatured form, but since this is not comparable to pepsin, this determination is of little value.

Optical rotation:

See table.

Carbohydrate was found by Herriot (1938) to constitute less than 0.1% of the purified pepsinogen and pepsin preparations or less than 0.2 ml. of hexose per ml. of protein and therefore is probably not an intrinsic part of the protein molecule.

CONVERSION OF PEPSINOGEN TO PEPSIN

Pepsinogen is converted to pepsin in solutions more acid than pH 6.0. (Herriot (1938) studies this conversion from two points of view - the kinetic and the chemical.

Kinetic study

In the kinetic study it was found that between pH 4.5 and 5.0 the course of the reaction is accurately described by simple autocatalytic equation. This simply means that a product of the reaction, in this case pepsin, catalyzes the reaction and therefore produces more of itself. The reaction is at least partly autocatalytic at pH 6.0 (incorrectly quoted as 0.0 in Herriot's 1938 paper) as shown by an increase in rate of conversion upon adding pepsin.

The conversion reaction rate varies enormously with pH, being hardly perceptible at pH 6.0 and relatively rapid at pH 4.0, i.e. 1 mg. protein nitrogen per ml. at 25°C. activates in about thirty minutes. Near pH 1.0 the rate of conversion is maximal and nearly 100 times that at pH 4.0. In solutions more acid than pH 4.0 the process of reaction is not a simple autocatalytic one such as between 4.5 to 5.0, though it is partly autocatalytic as far as pH 0.0, as shown by the increase in conversion rates by the addition of pepsin. Below pH 1.0 the rate of activation decreases, apparently not due to any denaturing effect of acid on pepsin or pepsinogen. The presence of salts in the medium accelerates conversion, depending somewhat

on the charge or valency and the concentrations of the ions of the salt; increasing salt concentration increases the rate of reaction on the alkaline side of pH 2.0 but decreases it on the acid side of pH 2.0 (Northrop, Kunitz and Herriot). At pH 2.0 the conversion is practically instantaneous (Sumner and Somers 1943); the temperature coefficient of conversion of pepsinogen to pepsin is 2 (Ege and Menck-Thygesen 1933).

Chemical study of the conversion has shown that the pepsinogen molecule is broken down as pepsin is formed from it, 15 to 20% appearing as non-protein nitrogen, i.e. not precipitated by hot 2.5% trichloracetic acid. This nitrogen is not produced by the destruction of pepsinogen by pepsin, because further addition of pepsin to the solution does not produce any more non-protein nitrogen than in a spontaneous autocatalytic reaction. That this protein was not simply an impurity was shown by the parallel and linear increase of this fraction with the increase in peptic activity resulting from conversion of pepsinogen, and that both increase or stop together under varied conditions of testing.

It is believed (Herriot 1938) that this non-protein nitrogen fraction in part or as a whole acts as a dissociable inhibitor of pepsin with maximal activity at pH 5.8 where rennet activity is measured. At the acid pH of haemoglobin digestion the inhibitor is completely dissociated from the pepsin and therefore need not be taken into account. The conversion of a molecule of pepsinogen to pepsin releases about 9 amino groups by the peptide link rupture only caused by pepsin.

Herriot (1938) states "It appears, then, that upon acidification of a solution of pepsinogen, an enzymatically inactive, reversibly denatured protein with an isoelectric point of pH 5.8 is split at probably not more than 9 peptide linkages to produce pepsin, an active irreversibly denaturable protein, isoelectric at pH 2.7, and a non-protein nitrogenous fragment which contains 15 - 20% of the original pepsinogen nitrogen".

Proteolytic activity of pepsinogen.

This important point was studied by Herriot (1938). Since all determinations of peptic activity of pepsinogen are done at pH where pepsinogen is converted rapidly to pepsin, two estimations - rennet and gelatin viscosity are carried out at pH 5.8 and 4.7 where little pepsinogen is converted to pepsin within the time required for estimation.

The following table shows that pepsinogen is practically inactive proteolytically.

TABLE NO: 2

(a) Specific activities.

Materials	Methods.			
	Haemoglobin Hb (P.U.) mg.P.N.	Rennet Ren. (P.U.) mg.P.N.	Gel.viscosity Gel.V (P.U.)mg.P.N.	Edestin NPN Ed.Sol. (P.U.) mg. P.N.
5x Crystallised Parke Davis pepsin	0.21	475	13	0.50
5x Crystallised Parke Davis pepsin	0.22	410	18	0.44
2x Crystallised Cudahy pepsin	0.30	455	33	0.57
3x Crystallised Cudahy pepsin	0.26	470	33	0.55
Crystalline pepsin from crystalline pepsinogen	0.26	360	12	0.48
3x Crystallized pepsinogen	-	1	1	-

TABLE NO: 2

(b) Specific Potential activities of Pepsinogen preparations.

Materials	Methods.			
	Haemoglobin Hb. (P.U.) mg.P.N.	Rennet Ren. (P.U.) mg.P.N.	Gel. viscosity Gel. vis (P.U.) mg.P.N.	Edestin NPN Ed. Sol. (P.U.) mg. P.N.
3x Crystallised pepsinogen	0.20	575	17	0.42
Average of 10 preparations of pepsinogen	0.22	250 - 300	-	-
3 copper treatment but uncrystallised pepsinogen	0.21	270	17	0.46

Table from Herriot (1938)

Section (a) of the table shows the similarity between different samples of pepsin, whether derived from commercial or pepsinogen sources, and the inactivity of pepsinogen. Table (b) shows that this pepsinogen is potentially active, i.e. when converted to pepsin.

Inactivation of pepsinogen

Pepsinogen may be inactivated by heat, ultraviolet light, iodine and by pressure. (Northrop, Kunitz and Herriot 1948).

CHAPTER III

METHODS.

This chapter deals with theoretical and practical considerations involved in pepsinogen assay, the latter mainly concerned with the various methods employed, which are critically reviewed. Personal methods and experimental observations on these are then described.

Theoretical Considerations.

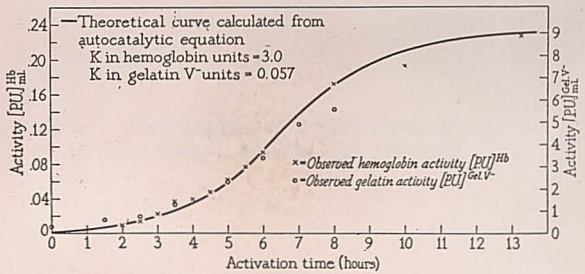
Pepsinogen is the inactive precursor of pepsin. This inactivity has been confirmed on the crystalline pepsinogen isolated in the pure form by Herriot (1938). It had been thought that at pH 4.5 pepsinogen did liquefy gelatin slowly, but it is known that at that pH pepsinogen is slowly autocatalytically transformed into active pepsin which is just detectably active at that pH (Northrop, Kunitz and Herriot 1948) and this activity, therefore, is of the pepsin so formed. Herriot has shown that at pH 4.7 pepsinogen does not liquefy gelatin and at pH 5.0 does not clot milk.

In order, therefore, to assay pepsinogen, one has to express the results in terms of pepsin, since it has to be changed into pepsin before showing any activity and then by

definition, at the pH of assay, it is pepsin. Therefore in speaking of pepsinogen assay, one in fact is dealing with pepsin assay, preceded by the conversion of pepsinogen into pepsin.

The only question one has to answer in that case is how rapidly pepsinogen is transformed into pepsin and whether a standard reproducible percentage is transformed into active pepsin (See also Chapter II). If the transformation is not immediate or complete, one would have to incorporate a suitable time and concentration of acid in the method to obtain standard results for pepsinogen estimation. The conversion into pepsin has been stated to be rapid at pH 1.0 to 3.0, slow at 3.0 to 4.0 and very slow above that (Gottlieb 1924), though these results should be interpreted with caution since he used the inaccurate fibrin method in that study. Ege and Menck-Thygesen (1933) have shown that conversion starts at pH 5.1, and is ten times more rapid at pH 4.1. At pH 2.0 it is instantaneous (Sumner and Somers 1943). Herriot (1938) states that conversion of crystalline pepsinogen commences at pH 6.0 and is increasingly rapid at lower pH (see Chapter II).

The accompanying graph shows that the conversion to full activity (0.22 $P.U._{Hb}^{\cdot}$ /mg.N) is not complete at pH 4.6 for about 12 hours.



Conversion of 1.0 mg. pepsinogen nitrogen per ml. into pepsin at pH 4.6 and 25°C.

Fig. 3. Conversion of crystalline pepsinogen to pepsin (Northrop, Kunitz and Herriot, 1948)

This graph further shows that the experimental result follows an expected autocatalytic reaction

$$\frac{dA}{dt} = KA\,(Ae - A)$$

where A = activity at the time, t, and Ae is the final activity. The rate of reaction depends on the acidity, salt concentration and pepsinogen and pepsin concentrations, which may be summarised as follows (Northrop, Kunitz and Herriot 1948).

(1) The addition of pepsin or increasing the concentration of pepsinogen increases the rate of reaction under all conditions. The reaction is therefore essentially autocatalytic throughout.

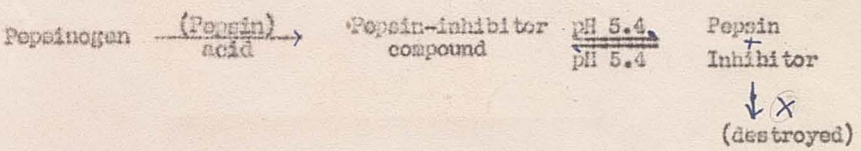
(2) The reaction is catalyzed slowly by hydrogen ions.

(3) The reaction has a maximum rate near pH 2.0

(4) Increasing the salt concentration increases the rate of reaction on the alkaline side of pH 2.0 but decreases it on the acid side of pH 2.0

(5) In solution more acid than pH 4.5 or in the presence of high concentration of salt, the pepsin appears in solution in combination with an inhibiting substance derived from part of the pepsinogen molecule. This compound dissociates slowly in solutions more acid than pH 5.4 into free active pepsin and the inhibitor. On long standing with pepsin, the inhibitor is destroyed.

The entire reaction, therefore, takes place as follows:

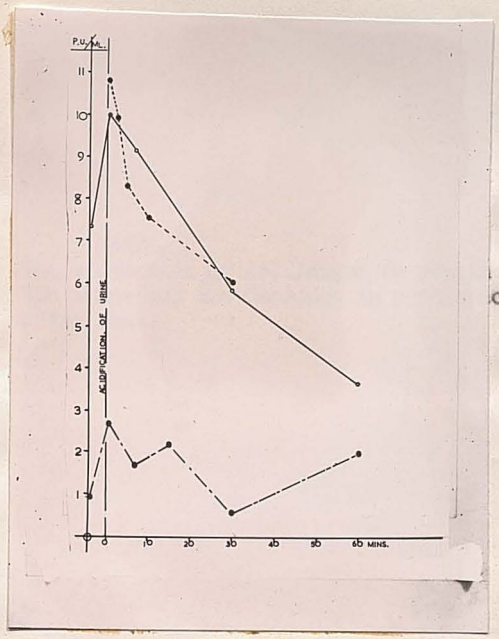


For practical purposes, therefore, one may assume that near pH 2.0 pepsinogen is instantaneously converted into pepsin (Sumner and Somers 1943).

Experimental observations.

These deal with an attempt to show how rapidly the urinary pepsinogen is converted to pepsin, and how soon after that this pepsin is destroyed. The following graph shows the activity of

3 acidified urines up to 60 minutes after acidification to pH 2.0 by mixing 10 ml. urine with 2.5 ml. 0.045 N HCl.



in
ctivity

Fig. 4. The conversion of pepsinogen to pepsin in the urine and the decrease in peptic activity after that.

It appears from these figures that the conversion to pepsin is rapid at pH 2.0 but that after acidification a rapid decline in the concentration of assayable pepsin occurs. The similarity in curves between urines 1 and 2 and the difference between those and urine 3 is probably explained by the difference in concentrations of pepsinogen, and possibly one might assume a difference in salt concentration between these urines, that is that with higher concentrations a stronger concentration of inhibitor is formed, or rather that at low concentrations of pepsinogen (and presumably other substances) in dilute urine, little or no inhibition of

pepsin occurs. That the conversion is complete or maximal is not certain, though probable. It is therefore essential to first acidify the urine and then digest as soon as possible after mixture with the HCl.

Having established, rather unsatisfactorily, it must be admitted, that pepsinogen is rapidly autocatalytically (and irreversibly) transformed into pepsin at pH 2.0, and this presumably maximally, the rest of the discussion will be concerned with the assay of the pepsin so formed from pepsinogen.

Before proceeding to a discussion of different methods, these methods should be examined in the light of the kinetics of enzyme action. There are three main techniques applicable to enzyme measurement, which fit the picture of a two phase reaction (Van Slyke 1942).

Technique I Activity measurement in substrate concentration high enough to give maximal rates.

This means that there should be sufficient substrate to allow the enzyme to act at maximal speed, and only the initial speed of the reaction is measured. If this period is short enough the amount of substrate split will be proportional to the amount of enzyme present. This period must be long enough, also, to allow of accurate timing and measurement of the substrate decomposed, but not so long as to allow sufficient of the substrate to be destroyed so that the enzyme will have to work at only part of its maximal speed. A short period of 5 to 60 minutes has several advantages,

(a) it limits the amount of enzyme decomposed so that the

concentration remains high enough to support maximal activity,
(b) the short reaction limits the accumulation of end-products which might retard the action of the enzyme,
(c) it minimizes the chance of inactivation of the enzyme under conditions of the test.

This is the technique employed in peptic assay with the haemoglobin method and is emphasized later in this chapter (p. 61)

Technique 2. Activity measurement in substrate solutions so dilute that the monomolecular law is simulated, on applying the die-away monomolecular formula and its constant will vary directly with the amount of enzyme present. This is only advantageous where the substrate is relatively insoluble.

Technique 3. One may use the general law that with a given substrate the time required to decompose a given fraction will vary in inverse proportion to the amount of enzyme present. This is the basis of Mett's method (p. 42). This applies to either Techniques 1 or 2. It also holds, regardless of how the products affect the enzyme, since conditions of digestion are the same if the same amount of substrate is hydrolyzed. One condition of failure is that where the enzyme loses its activity during the reaction. Where a relatively inactive enzyme would take so long to digest a fixed amount of tissue that it would become inactive, the enzyme might disappear before the expected digestion was complete. This method, therefore, depends on stability of the enzyme.

Furthermore, catalysis by definition affect only the velocity of a reaction and the activity of a catalyst, therefore, should be expressed as a velocity constant. Since enzymes are generally considered to be catalysts, the unit of enzyme activity should also be expressed as a velocity constant. In order to do this, however, it is necessary to know the kinetic equation for the reaction. In the case of most enzyme reactions, this equation is not known and it is therefore not possible to express the activity of the enzyme as a velocity constant.

Therefore, an arbitrary definition of activity has to be used and for pepsin it is convenient to express activity in terms of the "initial rate of reaction due to the presenceof the enzyme" (Northrop 1932). This definition is useful for analytical work and may be used to compare the activity of various quantities of the same enzyme with the same substrate, but cannot be used to compare the activity of different enzymes acting on different substrates. Proteolytic activity, as defined this way, corresponds to the measurement of optical activity and refers to a property of the solution (Northrop 1932).

PRACTICAL CONSIDERATIONS.

These techniques and this definition should therefore be applicable in some way to the various methods employed for peptic assay. In addition to these considerations, the yardstick of practicability should be applied to any method as examined.

It would perhaps be best at this stage to list the various methods that have been employed in peptic assay before proceeding to further discussion on them.

DATE	AUTHOR	METHOD.
1778	Stevens	Meat liquefied by gastric juice.
1839	Wasmann	Discovery of pepsin-digestion of meat and egg-white.
1861	Brücke	Fibrin flakes, egg-white.
1881	Grützner	Solid fibrin.
1886	Gehrig	Carmine stained fibrin.
1894	Mett	Egg-white tubes.
1894	Hammerschlag	Egg-white precipitation with Esbach's reagent.
1907	Fuld and Levison	Edestin
1907	Jacoby and Solms	Ricin
1908	Gross	Casein
1909	Liebmann	Egg-white suspension.
1921	Michaelis	Serum proteins
1922	Glaessner	Globin (of Hb) with precipitation
1922	Ege	Edestin modified with titration method.
1922	Loeper and Debray.	Blood proteins, for blood enzymes.
1922to 1932	(Euler) (Willstatter) (Northrop) (	(Rennet (Gelatin viscosity changes (Formation of soluble nitrogen (Increase in formol-titration.
1932	ANSON AND MIRSKY	HAEMOGLOBIN

The methods described up to 1922 can be divided into two groups (Glaessner 1922)

(1) Those depending on the digestion of solid proteins, and

(2) Those depending on the precipitation of protein in solution.

1. Methods depending on the digestion of solid proteins.

(a) Brücke (1861) who used the clearing of suspensions of egg-albumin at room temperature or of fibrin flakes at 36°- 40°C and was the first to detect peptic activity in the urine which he acidified, on collection, with phosphoric acid. The egg white method was later adopted by Liebmann (1909).

(b) Grützner (1892) who digested solid fibrin, and who

(c) with Sahli and Gehrig(1885 and 1886) modified by staining the fibrin with carmine and measuring the amount of dye released after digestion. The fibrin was preserved in glycerin from which it was removed prior to use and mixed with equal amounts of 0.1% HCl before addition of the gastric juice. The disadvantages of the method are the inequality of different samples of carmine-staining of the fibrin, the long time necessary for estimation and the difficulty of the method. It might be sufficient for gross work but is useless for accurate or comparable investigations and has long since been abandoned.

(d) The method of Mett (1894) modified by Nierensteiner and Schiff (1902) in which glass tubes are filled with coagulated egg-white and suspended in the acidified test solution. This method is still in fairly wide clinical use and has the advantage of being

easy. However, the disadvantages such as gross inaccuracy at high pepsin concentrations, the vague end point and the question of the accuracy of Schutz's law by which the results are worked out, serves to discredit this method for any but the grossest clinical use.

(e) Jacoby and Solms (1907) who depend on the clearing of a fine suspension of ricin/egg white. This method falls down on the difficulty of preparing a reproducible substrate and of the inherent difficulty in measuring losses in translucency of a suspension in which the particles are not entirely uniform.

(f) The method of Michaelis (1921) who used suspensions of diluted human (12x) or sheep serum (15x) precipitated with 10% sulphosalicylic acid to produce a milky suspension has the same objections as the previous method and is just as tedious.

The objection to all these methods (except Mett's) is that they are all tedious and including Mett's, were inaccurate because of difficulty in preparing reproducible substrates and of accurately measuring end-points. None of these authors could measure accurately the pH of the digest and were therefore possibly digesting at non-optimal pH.

II. Methods depending on change in precipitation of dissolved proteins

(1) Hammerschlag (1894) who used the fact that egg-white in solution is precipitated by Esbach's reagent and loses this property on being digested by pepsin. The estimation takes a long time, the end-point is vague and one needs a large amount of gastric juice;

consequently the method did not survive for long.

(2) The precipitation method of Fuld and Levison (1907) in which Edestin in HCl is digested and the undigested portion precipitated with NaCl. This is then titrated with alkali (NaOH) and the amount of NaOH used to dissolve the precipitated Edestin measures the amount undigested. The objections to this method are difficulty in the preparation of substrate, inequality of samples and the vague end-point of solution of undigested Edestin.

(3) Gross (1908) who employed a similar principle to Fuld and Levison, but used casein in HCl, incubated with gastric juice for 15 minutes at 39-40°C.; undigested casein is precipitated by sodium acetate, the amount remaining being used as an index of peptic activity by subtraction. Objections to this method are that pure casein is not easy to keep and impure casein does not give a clear solution or a sharp end-point. Gross (1906) commented on the Schütz-Borrisow law (1902) which states that pepsin activity is proportional to the square of the protein digested. He showed that peptic activity is mathematically proportional to the amount digested.

(4) Glaessner (1922) employed the globin of haemoglobin which is precipitated with ammonia and redissolved with NH_4Cl as a measure of the amount left over after digestion.

The objection to the second group of methods is the tedious nature of the method - where ten to twenty tubes of various dilutions of test substances are employed, that at best 80% accuracy is

possible, and that the judgment of end-points of opacity of suspensions as precipitates is difficult.

(5) Ege (1923) improved the method of Fuld and was the first to measure the pH of his digest. He used 10 ml. edestin in acetic acid at pH 1.5 to 1.6, with 1 ml. of test fluid and precipitated with up to 5 ml. of 20% NaCl, followed by 70% $(NH_4)_2SO_4$ and in a comparison chamber, compared this to a standard 0.5% gum-arabic suspension. The end-point was reproducible to within 1 - 3 drops of precipitant. This method was further refined by comparing the unknown with standard Armour pepsin. The method was later used by Gottlieb (1924) who claimed an accuracy of 95%, and remained in general sue for scientific work until the haemoglobin method of Anson and Mirsky (1932) superseded it.

The objections to all these methods may be briefly recapitulated. All the methods except Mett's (1894) and possibly Liebmann's (1909) are tedious and impractical; the advantage of simplicity in the latter is cancelled by their inaccuracy, and the others, except that of Ege (1923) are inaccurate as well as tedious. Substrates of most of these methods are difficult to prepare and are not reproducible. The latter fault is perhaps their greatest drawback. The end-points in all these methods depend on nephelometric measurement and as such, require so many observations to be accurate, as to invalidate it as a principle for comparative measurement.

All the authors, except Ege (1923) and Gottlieb(1924) did not measure the pH of the digest and one must assume that many of their

observations were on digests that were not at optimum pH and therefore not comparable. All the methods quoted, except that of Cross (1908) employed long incubation times and an incubation which is allowed to proceed up to 120 hours (Gottlieb for serum pepsinogen) is open to serious criticism, as are many that were incubated for 12 - 36 hours, for gastric juice or urine (see p. 61)

Modern Principles of Peptic Assay

The various principles and techniques involved in peptic assay are discussed by Northrop (1932) under three headings,

(i) the measurement of changes in viscosity of protein produced by pepsin,

(ii) the production of non-protein nitrogen by pepsin and the determination of activity by the increase in formol titration. This introduces a new orientation towards methods of peptic assay and at the same time a rational approach to the action of pepsin on protein solutions.

(iii) the production of amino(carboxyl) groups.

The first effect of pepsin on a protein solution is a rapid decrease in viscosity of the solution, the second a slower decrease in substrate protein nitrogen and thirdly, the slowest of all, an increase in the number of titratable carboxyl(amino) groups. Other changes which occur, but are less specific, are in the optical acitivity and in conductivity. The three former

are the more characteristic of changes in a protein solution.

I. Viscosity changes.

Viscosity changes may be measured easily and rapidly and accurately (error of 3%) (Northrop and Hussey 1923). The disadvantage is firstly that of preparing comparable protein substrates, though gelatin is a fairly constant substrate; and secondly, and perhaps more important, that the significance of viscosity changes and their relation to the chemical changes are obscure.

The proteins used by Northrop (1932) in determining viscosity changes are gelatin, edestin, casein and dried milk (for the casein solution) [illegible] The details of each method and a discussion of the technique are described in that paper. He claims that the results are proportional to the concentration of enzyme, though the results are for one concentration only and are proportional to time in that study.

Protein V.

The arbitrary units for this method, designated (P.U.) are defined as follows - one unit of activity is defined as a change in the specific viscosity of the standard protein solution at 35.5°C. at the rate of 1 per cent per minute. The quantity of material possessing one unit of activity is that quantity which will cause this rate of change in the viscosity when contained 5.2 ml. of the standard digestion mixture. The

choice of percentage is because this value is independent of protein concentration and that of the temperature of 35.5°C is because at only that temperature is gelatin viscosity stable.

For the coagulation of milk($(P.U.)^{Ren}$) the initial slope of the curve is not straight and the percentage increase in viscosity is determined arbitrarily from the point at which the specific viscosity is doubled and is obtained by dividing 100 by the number of minutes required to allow of this change.

II The productionnof Non-protein Nitrogen.

The production of non-protein nitrogen is the second method of determining peptic activity. It is not clear what the exact significance of these changes is in relation to chemical changes and has the disadvantage of being somewhat slower than viscosity change measurements and of the fact that there is no simple relation between the amount of non-protein nitrogen produced and the amount of active enzyme, except when the latter is very small and then the determination is inaccurate.

This determination is made with casein and edestin only where the results are proportional for low concentrations of enzyme or in the initial part of the digestion. Two methods are available - to measure the increase of non-protein nitrogen after different time intervals with a fixed concentration of enzyme; or to measure the increase of non-protein nitrogen after a fixed time with varying concentrations of enzyme. It is

usually necessary to measure more than one concentration of an unknown enzyme solution because the curves obtained, and interpreted by interpolation, may vary considerably with the purity of solution and specific activity must be independent of the quantity of the enzyme used.

In this method, one unit of activity ($P.U._{Prot.S}$) is defined as the liberation of non-protein nitrogen at the rate of 1 m.Eq. per minute at 35.5°C. in 6.0 ml. of standard digestion mixture by that amount of enzyme contained in 6.0 ml. of the digestion mixture, and is obtained from curves of either of the two methods mentioned in the preceding paragraph.

III. The production of amino(carboxyl) groups.

The production of amino (carboxyl) groups has the advantage of having a clear chemical relation to enzyme action, but the amount produced is small and as with the former method, not simply proportional to the amount of enzyme present. It is also rather inaccurate and rather difficult.

In this method, gelatin, casein and edestin are used and curves produced as before, activity being determined from the initial slope of the curve. With the same substrates the error is ± 5%, but with different samples the error is often greater and unpredictable.

One unit of activity ($P.U._{Prot.F}$) by this method is defined

as an increase in the carboxyl (or amino) groups in 6.0 ml. of the standard digestion mixture at 35.5°C. at the rate of 1 m.Eq. per minute, caused by that amount of enzyme contained in this mixture.

However, as much as these methods form a serious attempt at measuring some action of pepsin, they do not approach the ideal which should contain factors for all the various conditions affecting the activity of the enzyme so that a constant figure for the activity would be obtained, no matter how the determinations were carried out. While it is difficult to correlate all the differing factors affecting peptic digestion and impossible to connect all in one system of absolute units, the haemoglobin method of Anson and Mirsky (1932) has several advantages over other methods.

These are:- accuracy of measuring the end products, rapid digestion by pepsin giving proportionality of results for both concentration of enzyme and time within limits of extremes, and admirable reproducibility of substrate, ability of the substrate to keep for long periods unchanged, and the fact that small variations in conditions of the test do not influence the result.

The haemoglobin method was therefore chosen for use in this study because its theoretical and practical superiority over other methods of peptic assay.

PERSONAL METHODS.

Basic principle: The basic principle of all the methods is adapted from the original method described by Anson and Mirsky (1932) and later modified by Anson (1938). This method was chosen in preference to other methods because it is practical, accurate and reproducible and it was found that the method could be applied to all fluids in addition to those already described for urine (Bucher 1947) and for gastric juice (Beazell, Schmidt, Ivy and Monoghan 1938). (Farnsworth et al 1946).

The principle of the method is the digestion of haemoglobin at pH 1.5 to 2.5 by the test fluid for a fixed period and the measurement spectrophotometrically of the increase in trichloracetic soluble tyrosine-like substances with Folin Ciocalteau reagent in the non-protein portion of the digest.

In practice 5.0 ml. of a 2% acidified haemoglobin substrate (0.1 G.) is incubated with 1.0 ml. of the test solution for 10 minutes or an hour at 37°C. and at pH 1.7 to 2.2; the reaction is stopped with 10 ml. of 0.3 M. trichloracetic acid. A parallel blank is performed by adding the test fluid to the trichloracetic acid first to precipitate the enzyme and this is followed by 5 ml. of the substrate. Each is filtered (Whatman No. 1) and then dealt with similarly. To 2.0 ml. of filtrate is added 1.2 ml. of freshly diluted Folin Ciocalteau reagent 1:3 and then with shaking 4.0 ml. of 0.5 N NaOH. A blue colour develops which

is read between 5 and 20 minutes in a spectrophotometer at 560 mu. The difference between blank and incubated specimen is compared to a standard aqueous solution of l-tyrosine containing 2×10^{-4} m.Eq.(.0362 mgm.) per 2 ml.

Calculation $\frac{ER}{ES} \times CS \times \frac{VD}{VF} \times \frac{DE}{1} \times \frac{10"}{I.T} = P.U.$

Where ER – Extinction factor difference between blank and incubated filtrates.

ES – Extinction factor for standard.

CS – Concentration of standard in 2 ml. in m.Eq. of tyrosine(=2)

VD – Volume of digest and precipitant.

VF – Volume of filtrate

DE – Dilution of enzyme solution before adding to digest.

10" – Ideal 10" incubation time to conform to definition

IT – Actual incubation time in minutes.

Definition of Unit One unit of peptic activity is that amount of enzyme which will produce the spectrophotometric equivalent of 1×10^{-4} m.Eq. of l-tyrosine from a standard digestion of 0.1 G. Hb. in 5 ml. by 1 ml. of enzyme solution acting at 37°C. for 10 minutes, at pH 1.5 to 2.5.

Modification of the basic principle is determined only by the pepsin or by the pepsinogen content of the test fluid and its physical properties.

METHODS USED

(a) GASTRIC JUICE.

The juice is filtered through a small wad of cotton wool in a 20 ml. glass syringe and then diluted 1/50 in distilled water. Of this 1 ml. (≡ 0.02 ml. of gastric juice) is added to the 5 ml. of Hb. substrate and incubated for 10 minutes, then precipitated with 10.0 ml. of 0.3 N. trichloracetic acid.

(b) URINE

Since the enzyme in urine is pepsinogen, it has to be converted to pepsin before assay. The urine is therefore acidified by adding 2.5 ml. of 0.33 N. HCl. to a 10 ml. aliquot of urine. The resulting pH was found to be between 1.5 and 1.8. The conversion is very rapid and the subsequent loss of pepsin also fairly rapid (p. 37) so that this mixture should not be left standing for any avoidable time before 1 ml. of this is added to the substrate. The urine is incubated for 10 minutes and precipitated by 10 ml. of 0.3 N. trichloracetic acid.

(c) PLASMA (OR SERUM)

This method for plasma pepsinogen has not been described before.* This fluid presented certain difficulties in the assay. Firstly, the enzyme content is very small - about 1/20 of urine pepsinogen, and the increase in tyrosine-like substance, therefore, very small. This, however, is partly balanced by the absence of colour producing substances in the plasma (i.e. a pale blank) so

* Mirsky et al described another method in July 1952.

that smaller differences are more easily detectable. However, the incubation time used is 60 minutes, which is sufficient for all estimations and is a practical time. Because the colour produced in the filtrate is so pale, unnecessary dilution is further avoided by halving the volume and doubling the concentration of trichloracetic acid, therefore 5 ml. of 10% trichloracetic acid is used to precipitate the 6 ml. of digest.

Because of the small volumes and the high protein content prior acidification of the plasma with HCl is likely to produce errors in dilution and occasionally precipitation of protein, if dilution be avoided by using strong acid. It was felt that with the 60 minute digestion and the stability of the whole digest at pH 1.6 to 2.1. after addition of the plasma to the Hb., the pepsinogen of the plasma would be sufficiently buffered at an optimum pH to allow full conversion to pepsin necessary for the assay.

Therefore 1 ml. of undiluted plasma is added to 5.0 ml. of 2% Hb. at 37°C. and incubated for 60 minutes, and then precipitated with 5 ml. of 10% trichloracetic acid.

Another difficulty arose in the preparation of the blank. By adding the plasma to the trichloracetic acid before the substrate or after the substrate, a thick protein curd is produced. It was found that if the plasma and the substrake were shaken together first and this mixture precipitated immediately with the trichloracetic acid, a fine homogeneous precipitate is formed, as it is in the incubated specimen.

After filtration, the filtrate is dealt with as with the other fluids.

(d) OTHER FLUIDS

Using the method as described for plasma pepsinogen other fluids were assayed for pepsinogen. These included cerebro-spinal fluid, sweat, pleural fluid, ascitic fluid, oedema transudate, whole blood, white cells and pus. All have a similar content to plasma and are similarly incubated for 1 hour and precipitated with 5 ml. of 10% trichloracetic acid, except white cells which may be incubated for 10 minutes because of their high content of pepsinogen.

EQUIPMENT

1. Unicam spectrophotometer with modified 12-volt circuit.
2. Cambridge glass-electrode pH meter.
3. 37°C. water bath.
4. 5" x 1" test tubes.
   5" x ½" test tubes.
   Pipettes.
5. Whatman's No. 1 or 42 filter paper.
6. Solutions:
   (a) Armour's haemoglobin substrate
   (b) 0.55 N.HCl and .067 N. HCl.
   (c) 10% and 5% (0.55 N.) trichloracetic acid.
   (d) Folin-Ciocalteau reagent.
   (e) 0.5 N. NaOH

SUBSTRATE

Haemoglobin forms a very suitable reproducible substrate for the estimation of proteinase digestion, mainly because different batches are digested at the same rate by a given protease solution and even when there is peptidase present in addition to proteinase, the formation of products not precipitated by trichloracetic acid is due, so far as is known, to proteinase alone (Anson 1938).

In this study, two different types of haemoglobin were used - firstly human haemoglobin from waste transfusion blood, and secondly bovine haemoglobin first described by Orringer, Lauber and Hollander (1950) supplied by Armour's Lted as "Lyophilized bovine haemoglobin enzyme substrate" *

The human blood used was supplied from the transfusion service and was prepared for use according to the method of Anson (1938). The blood was centrifuged and the supernatant serum siphoned off. The red cells remaining were washed three times, in cold normal saline, removed each time by siphoning after centrifugalization. The cells were then dialyzed in $\frac{1}{2}$" Dupont cellophane against running cold tap water for 24 hours to remove colour producing substances. The red cell remnant was then partly dehydrated in vacuo to give a stock solution of about 20 G.%. This was kept frozen solid at -10°C. and remained fresh

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* Armour's Ltd. kindly supplied free of charge the first 15 Gms. used in preliminary studies.

for about 2 months. It was found to be too tedious to freeze-dry large amounts to produce the more stable haemoglobin powder.

The Armour's haemoglobin powder was made up as a 10 G.% stock solution every 10 days or so and kept frozen as -10°C.

For assay use, the stock solution is thawed immediately before use, and made up as a 2% solution in .067 N.HCl. This should be fresh at least every day. The figure for the strength of the diluting acid was arrived at when it was shown that this dilution of haemoglobin acts as a buffer of moderate strength at pH 1.80 ± 0.2. This is the same for both human and bovine haemoglobin solutions which have pH 6.6 before addition of the acid and 1.7 and 1.8 respectively after acidification.

The addition of aqueous solutions of pepsin (1 ml. to 5 ml. of acid substrate) does not affect the pH and the addition of acidified urine (which in the proportion of 2.5 ml. of 0.43 N.HCl to 10 ml. of urine in a large number of urines reduces the pH to between 1.5 and 1.8, which is the same pH as substrate) does not affect the pH of the digest mixture to any extent. The addition of 1.0 ml. of plasma or serum to 5.0 ml. of substrate raises the pH of the digest by between pH 0.2 and 0.4. Thus for all assays in which the pH was measured, the lowest was 1.45, the highest 2.2. This is well within the limits of action of pepsin (1.5 to 2.5 - Northrop, Kunitz and Herriot 1948) and from numerous samples taken, it can be assumed that all assays were made in that pH range.

INCUBATION TIME

This poses the question as to whether there is arithmetical proportionality of digestion by time at a constant pH. That this is so, and that the Schutz-Borisow law (1900) which states that peptic digestion is progressive by the square root of the time or concentration, is not valid (Gross 1908) has been adequately shown in the digestion of haemoglobin for pepsin solutions, for gastric juice (Gross 1908) and for urinary pepsinogen (Mirsky et al 1948).

EXPERIMENTAL OBSERVATIONS

However, because this haemoglobin method depends for accuracy on an excess of substrate (see p. 38) it is important that the enzyme does not digest all of the substrate within the time of digestion. In order to show the linear proportionality of pepsin digestion of haemoglobin and to determine the upper limits of both this strength of pepsin and the time of digestion for the substrate as used in these experiments (0.1 G. in 5 ml.) various strengths of Armour's 1:10,000 scale pepsin were used to digest standard haemoglobin for varying lengths of time.

From Graph 5 (1,000 µg pepsin/ml.) it will be seen that the maximum amount of digestion occurs at 1 hour and 26 hours digestion produces no more of the products of digestion. This figure in one experiment is 282 Tu (15th June 1951) and in another (22nd June, 1951) 262 Tu. per 0.1 G. haemoglobin substrate. It can further be seen that proportionality holds only up to about 30 minutes,

whereafter the slope tails off very rapidly into the horizontal which commeness at 1 hour. The initial slope of the curve is very steep and therefore at high concentrations of enzyme compared to the comparatigely dilute substrate, linear proportionality is probably not valid, even in a short period of digestion. In the experiment using 100 ug. pepsin/ml., satisfactory linear proportionality between time and digestion was measured up to 2 hours. By extrapolation the limit of digestion (282 Tu per 0.1 G. Hb.) would be reached at about 2 hours 20 minutes (see graph).

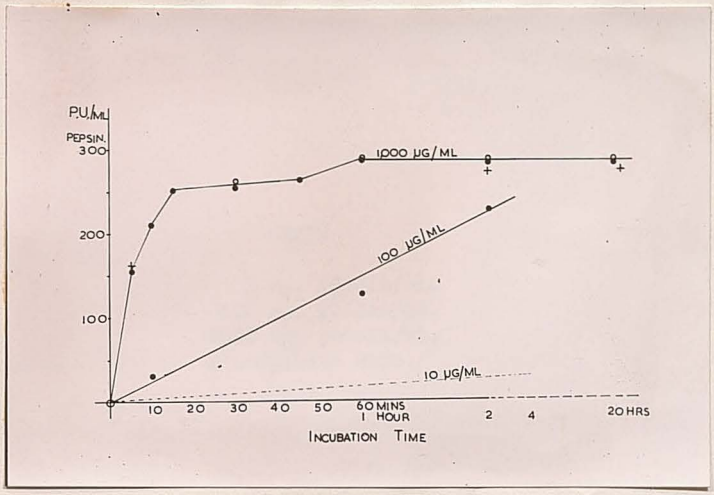


Fig. 5. Haemoglobin digestion with varying strengths of crystalline pepsin.

5

In the third experiment using 10 ug/ml. pepsin, satisfactory linear proportionality existed within the 2 hour incubation period. By extrapolation the maximum digestion time of this concentration of enzyme for the standard digestion system is 18 hours, when 280 Tu. would be produced. (See graph).

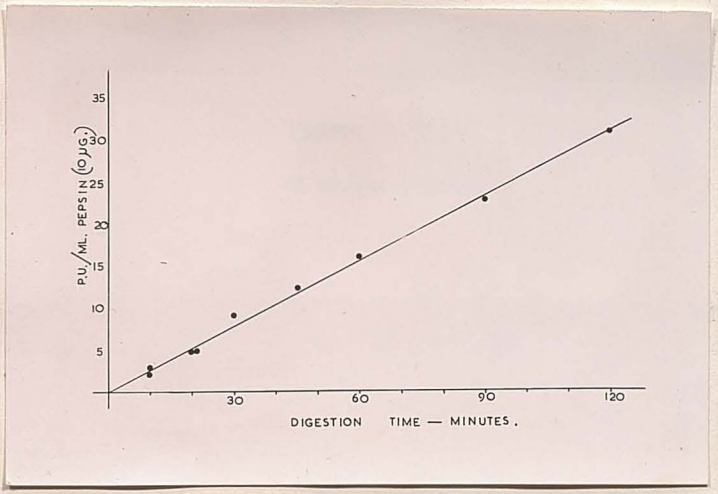


Fig. 6. Digestion of haemoglobin by weak pepsin (10 ug./ml.)

The normal urinary content of pepsinogen lies somewhere between the pepsin equivalent limits of the 10 ug/ml. and the 100 ug/ml. pepsin; that of gastric juice usually over 1 mg., often up to 6 mg/ml. of juice.

CONCLUSION

The digestion of haemoglobin by urine having a pepsinogen equivalent of between 10 and 100 ug./ml. should never be allowed to exceed 2½ hours (see above), and gastric juice should be diluted, unless apeptic, by at least 50 times. 10 minute incubation times were found to be convenient and capable of accurate measurement and allowing of simultaneous incubation of up to 20 specimens. This is done by starting successive tubes at ½ minute intervals and precipitating them at the end in the same rotation. For low concentration pepsiongen solutions (plasma (or serum) and body fluids, other than urine or gastric juice), 1 hour was found to give more accurate results.

COMMENT ON TIME OF DIGESTION.

Mirsky et al (1948) used 10 minute incubation times for urine, but later (1950) stated that 30 minutes was a more satisfactory time for incubation. Bucher (1947) used 1 hour, and Farnsworth et al (1948) 18 hours. Older authors, using other methods, used incubation times of up to 120 hours. In the older methods prolonged incubation times were merely an expression of the coarseness and inaccuracy of their methods. While Farnsworth's digestion period was probably accurate enough for apeptic urines, one must regard his estimations of normal urines with some suspicion.

Theoretically, there can be no objection to any haemoglobin method (using 0.1 G. Hb.) which employs a digestion time of between 10 and 150 minutes for the assay of all solutions containing a

pepsin equivalent of less than 100 ug./ml. 1:10,000 Armour scale pepsin or its equivalent. Further, since there is a simple arithmetical proportion the results of any period of digestion within those limits can be expressed in terms of any other time-units; in other words, x units as assayed at 60 minutes $\frac{x}{6}$ 10 minute units or $\frac{x}{2}$ 30 minute units. This assumption allows one to compare two different incubation times, say 1 hour plasma pepsinogen and 10 minute urine pepsinogen in estimating renal pepsinogen clearance.

That one may assume a linear proportionality to exist for plasma pepsinogen may be seen from the graph below demonstrating this fact. For this experiment, the plasma of a uraemic patient (500 mg.% blood urea) was used in order to get reliable readings for short incubation periods.

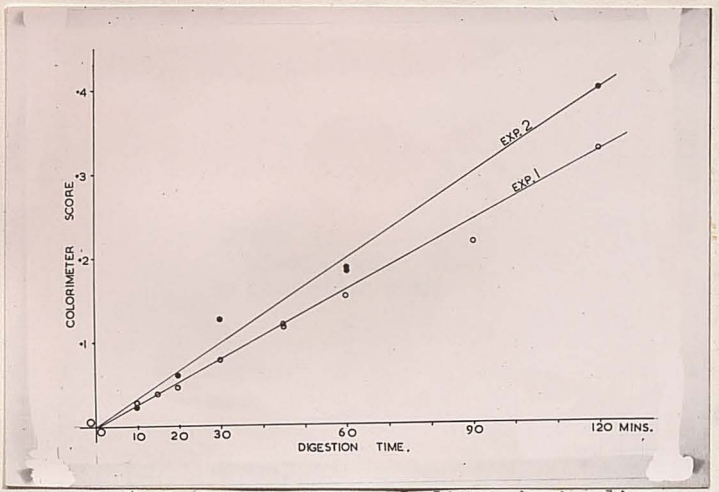


Fig. 7. Proteolytic activity of plasma showing linear proportionality of digestion with incubation time.

MODIFICATION OF VOLUMES USED.

The methods in use for determination of the tyrosine equivalent of the filtrate while being apparently correct and reproducible, require large volumes of reagent and are therefore tedious for routine use.

Anson (1938) recommends the addition of 10 ml. 0.5 M. NaOH and 3 ml. of diluted Folin Ciocalteau reagent to 5 ml. of filtrate. This makes a total of 18 ml., which has to be mixed either in an Erlenmayer flask (25 or 50 ml.) or in a 6" x 1" tube. Consequently only a few can be done at a time if both incubate and blank are to be measured simultaneously.

EXPERIMENTAL OBSERVATIONS.

It was found that by keeping the proportions of filtrate, NaOH and Folin-Ciocalteau the same, viz. 5:10:3, but reducing the total amounts to 2:4:1.2 in ml., a whole row of up to 12 incubations could be dealt with using a rack containing 24 3" x $\frac{1}{2}$" tubes. It was further found that there was some difference between adding the phenol reagent before or after the NaOH to the filtrate. If Folin is added last, the blue colour develops a little slower and fades sooner. The following table shows the comparison between adding Folin-Ciocalteau before and after NaOH to standard tyrosine. 5 different pairs of observations were made at 2 minute intervals.

2 m.Eq./2 ml.		4 m.Eq./2 ml.	
NaOH last	Folin last	NaOH last	Folin last
.224	.225	.450	.390
.224	.226	.455	.446
.230	.228	.460	.442
.230	.229	.452	.441
.222	.205	.460	.430

TABLE NO: 3

THE EFFECT OF ADDING FOLIN REAGENT AND NaOH IN A CERTAIN ORDER TO THE FILTRATE.

Another advantage of using 2 ml. of filtrate is that if thereshould be an accident, and the filtrate lost, another tube can be set up, whereas a second 5 ml. may not be ontainable if the large quantities be used.

This is of special advantage in the plasma estimation where the total quantity of digest is 11 ml., and total filtrate rarely more than 7 ml.

To determine how soon the blue colour evolves to a maximum and how soon it declines, the various concentrations of tyrosine in 2 ml. water were made up with 4.0 ml. 0.5 M. NaOH and 1.2 ml. of Folin-Ciocalteau reagent and measured at intervals from the time of mixing, at 560 mu. (Tables 4 and 5). While the

percentage decay is similar in all, the absolute decay in colour is proportional to the initial concentration of tyrosine (Table 4)

Concentration Tu./2 ml.	Fall.	% fall of max. colour
0.5	.015	12.6
1.0	.023	12.2
2.0	.029	11.1
3.0	.041	11.3
4.0	.054	11.5

TABLE NO. 4 RELATIVE AND ABSOLUTE VALUES OF DECAY OF BLUE COLOUR IN 1 HOUR.

TABLE NO. 5 SHOWING RATE OF EVOLUTION AND DECAY OF BLUE COLOUR IN 1 HOUR
(Colorimeter values.)

TYROSINE	MINUTES																	
TU/2 ml.	1-3	-5	-8	-10	-12	-15	-17	-19	-21	-25	-26	-28	-34	-39	-44	-47	-54	-60	% Decay in 1 hour
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0.5	.064	.066	.065	.063	.062	.062	.061	.061	.059	.059	.057	.056	.054	.054	.052	.052	.051	.051	12.6
1.0	.118	.118	.118	.112	.114	.112	.113	.111	.109	.110	.110	.107	.104	.100	.100	.100	.095	.095	12.2
2.0	.253	.254	.252	.251	.250	.250	.249	.248	.240	.242	.242	.240	.238	.234	.232	.229	.226	.225	11.1
3.0	.326	.328	.324	.321	.321	.321	.320	.318	.316	.314	.312	.309	.308	.305	.297	.293	.290	.289	11.3
4.0	.442	.446	.440	.439	.436	.438	.438	.434	.431	.430	.430	.426	.421	.415	.408	.403	.395	.391	11.3

From these results it will be realized that the blue colour develops maximally almost immediately - in about 5 minutes, and then fades proportionately to the original value. This difference in absolute fall should be remembered when two solutions for comparison are kept more than 10 minutes before reading in the colorimeter.

Another experiment to determine the colorimeter value for the 2 TU./2 ml. standard in 9 estimations is given below. They were all prepared in order 1 - 9 together with the water blank and read at 1½ minute intervals from time of mixing, accounting for the decline in the readings and showing the effect on the Es-B readings of delaying reading.

TIME (mins.)	BLANK	STANDARD (ES)	ES - B
0	.030	.238	.208
1½	.024	.242	.218
3	.025	.243	.218
4½	.020	.239	.219
6	.020	.241	.221
7½	.020	.240	.220
9	.019	.236	.217
11½	.016	.226	.210
13	.012	.226	.216
Average	.0204	.239	.216

TABLE NO. 6 MEAN VALUE FOR EXTINCTION VALUE OF STANDARD L-TYROSINE

Conclusions. The mixture should be made up as follows:- filtrate, Folin-Ciocalteau, NaOH in that order, and read within 10 minutes.

THE CHOICE OF SPECTROPHOTOMETER WAVELENGTH TO READ THE BLUE COLOUR PRODUCED BY FOLIN-CIOCALTEAU REAGENT ON TYROSINE-LIKE GROUPS.

Previous papers dealing with the subject do not mention the actual wavelength at which the final blue colour should be measured. Bucher (1947) uses filter No. 54 (540 mu). In order to determine the optimum wavelength, tyrosine solutions of varying strength in water were used. The tyrosine was made up in 5 ml. water to which was added 10 ml. of 0.3 M. trichloracetic acid, 10 ml. 0.5 NaOH, 1 ml. of 25% Na_2CO_3 and 3 ml. of 1:3 diluted Folin-Ciocalteau reagent. The following table shows the readings of duplicate specimens at various wavelengths for each concentration with the colorimeter set at 0 for the blank.

TABLE NO. 7 WAVELENGTH AND COLORIMETER READINGS IN THE LOGARITHMIC SCALE.

Concentration of Tyrosine in mEq. in 5 ml. of water.	680 mu	640 mu	600 mu	540 mu	520 mu	480 mu	440 mu	410 mu
0	0	0	0	0	0	0	0	0
4	.201 .202	.215 .205	.185 .190	.170 .170	.175 .145	.124	.103 .105	.100
8	.415 .411	.400 .395	.380	.355 .355	.300 .300	.252 .252	.222	.208
16	.77	.74 .74	.72 .72	.640	.560	.490	.415	.390
24	1.10	1.070	1.00 1.00	.930	.820	.700	.590	.550
32						.920	.760	.700

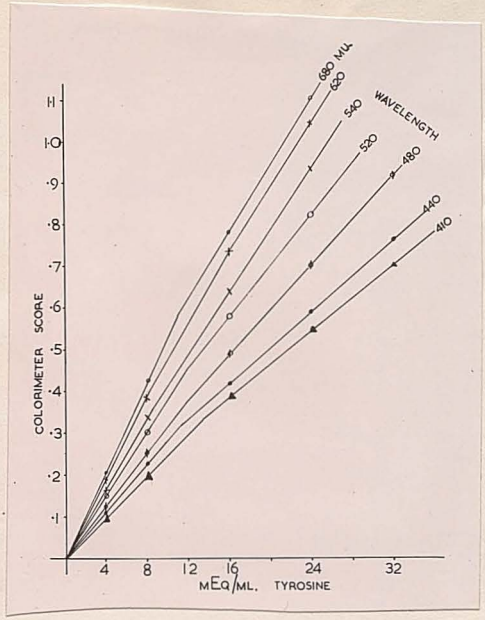


Fig. 8. Determination of optimum wavelength for reading blue colour (see text).

From the graph drawn from this set of results the conclusions reached are that for obedience to the Lambert-Beer law

1. Range 520 to 600 mu find the optimum 560 - 600 mu.
2. Absolute maximum concentration determinable 24 x 10^{-4} mEq. tyrosine
3. Maximum sensitivity is 8 - 12 x 10^{-4} mEq. tyrosine.
4. Avoid concentrations below E = 0.05 (≡ 0.5 x 10^{-4} mEq.)

Following these observations 560 mu was chosen as the standard wavelength for measurement of the blue colour produced.

DETERMINING THE DIFFERENCES BETWEEN BLANK AND INCUBATED SPECIMEN

At first, this difference was determined by subtracting the reading of the blank from that of the incubated filtrate, each being read against distilled water set at 0. It was found that this was not only tedious but unnecessary and when the blank was high, inaccurate as well, because the readings have to be made at the coarsely graduated left side of the scale. It was found that one can obtain the difference much more easily and with the exclusion of one step of the process by eliminating the distilled water zero. The colorimeter is set on the blank as near to zero as possible and the incubated specimen read against this reading. The difference is recorded as such directly. This is more accurate because the readings are mainly in the first half of the logarithmic scale, where the subdivisions are finer. If it should be necessary to read on the left side of the scale it is more accurate to read on the arithmetic scale, and from logarithm tables to convert this to logs, which will then give one a more accurate figure, rather than read directly the logarithmic scale of the machine.

COLLECTION AND STORAGE OF SPECIMENS.

URINE It has been adequately shown that the estimation of concentration of pepsinogen in the urine is not the ideal way to measure the excretion of this enzyme (Gottlieb 1924) and that for the sake of accuracy, results should be expressed as the amount of enzyme excreted in a unit of time, i.e. as a rate

of ecretion, either per hour or per 24 hours (Gottlieb 1924; Bucher 1947; Broh-Kahn et al 1948). The latter authors claim that any time of collection of 3 hours or more is representative of the whole 24 hour period and that one estimation per individual is sufficient. I would disagree with these authors, and believe that in some instances, collections of less than 24 hours are not representative for the 24 hours. In the case of an 18 year old female control (J.W.) it was found that while concentration remained comparatively steady, the volume of 12 hourly urines by day was far higher than the corresponding night urines.

Thus for 6 consecutive 12 hourly periods and the two following 24 hour periods:-

VOLUME OF URINE		CONCENTRATION (COLORIMETER SCALE)	P.U./Hr.
D.	860	.11	724
N.	350	.12	322
D.	660	.15	758
		25 mgm. A.C.T.H.	
N.	300	.06	137
D.	710	.05	270
N.	320	.06	146
24 hrs.	1120	.142	611
24 hrs.	1150	.155	684

TABLE NO: 8 THE DIURNAL VARIATIONS. IN URINE VOLUME AND PEPSINOGEN IN 1 PATIENT.

Following these results, it was concluded that only 24 hour urines could be relied on to express the true 24-hour excretion pattern.

As to Mirsky's (1948) statement that one estimation is sufficient for the individual excretion pattern to be determined, it was found that there was often such enormous variations from day to day as to make the estimation of at least 2 and usually 3 consecutive days collection mandatory. In one case (J.B.) on three successive days the rates of excretion were 1630, 322 and 728 units per hour. These readings were duplicated because of this wide variation and found to be the same. No obvious explanation could be given for this variation as the patient was in bed on a standard diet and fluid intake during the observation period, and no other tests were done on the stomach at the same time.

Generally, however, the test at different times on the same patient produced similar results and for most patients, two 24-hour urines collected during a standard diet period will suffice to determine the basic pattern of that patient's excretion.

In this series at least 2 and generally 3 urines were tested on each patient. The urines were collected in win chesters containing 10-15 ml. of toluol and refrigerated at + 4°C. as soon as collected.

The collection of specimens by various authors differ and

depends on what they believe is an adequate sampling time. Janowitz (1950) collects a 3 - 12 hour specimen fasting, resting or 5 - 8 hours after food. Bucher (1947) following Wilenko (1909), Takeda (1910) and Gottlieb (1924) collected 24 hour specimens, and Gray et al (1950) followed Bucher. Mirsky et al (1946) and Broh Kahn et al (1948) concluded from their investigations of the time of collection that any period of the day of over 3 hours was enough, since neither food, nor sleep nor activity had any effect on urinary pepsinogen excretion.

One must criticise the collection of cat's urine directly into an acid solution by Bucher and Ivy (1947) unless they are assayed within 24 hours - since Mirsky et al showed that at pH 1.5 pepsinogen in the urine is stable for 24 hours. I believe, however, that the urine should not be acidified until immediately prior to assay, since there is a rather rapid decline within the first few minutes of peptic activity (p. 37).

However, at alkaline or near neutral pH, the urinary enzyme is stable for long periods. Both Bucher (1947) and Mirsky et al (1946) have shown this. The following tables give these results.

TABLE NO. 9 "STABILITY OF URINARY PEPSINOGEN AT 50° C. FROM 1 - 30 DAYS. RESULTS IN COLORIMETER SCORES." (Bucher 1947).

DAYS.	1	2	3	4	5	6	7	Urine No.
	6.04	5.82	5.80	7.41	7.41	7.64	8.00	pH of Urine
1	59	-	68	5	10	4	54	
2	59	98	-	-	-	-	56	
3	57	95	-	-	-	-	50	
4	60	98	-	-	-	-	-	
8	-	-	-	-	-	-	-	
15	56	97	65	-	-	-	-	
20	-	-	64	-	-	-	47	
30	-	-	60	5	10	6	-	

TABLE NO. 10 "STABILITY OF URINARY PEPSINOGEN FOR 28 DAYS AT VARIOUS TEMPERATURES". (Quoted from Mirsky et al (1948).

DAYS STORED.	ROOM TEMP.	3-5°C.	Toluol	Peptic activity Y/cc.
0		x	No	78
0		x	Yes	74
1	x		Yes	82
1		x	Yes	75
7	x		Yes	78
14	x		Yes	75
28		x	Yes	74

One may therefore conclude that provided no heavy bacterial growth occurs and the pH is near neutral that the urinary pepsinogen is stable at 3 - 5°C. for up to 28 days, at room temperature for up to 14 days, and at 50°C. (F.) for 15 days at pH 6.1, and for 30 days at pH 7.4.

In this investigation, all urines were refrigerated upon collection and always assayed within 21 days, but usually within 7 days of collection.

SERUM OR PLASMA (See also p. 53).

For estimation of circulating pepsinogen, it had to be determined first of all whether there was any difference between the plasma and the serum content of a specimen of blood. Gottlieb (1924) believed that there was none, but also stated that he believed that pepsinogen was adsorbed on to fibrin but only at acid pH. Since in the clotting of blood to produce serum fibrin remains in the clot, it was important to be sure that this fibrin did not withdraw pepsinogen from the plasma.

One sample of blood from a uraemic patient (J.H.) was divided into two portions, one allowed to clot, the other anticoagulated with heparin. The results were 216 Tu % and 212 Tu % respectively for the serum and the plasma so obtained. This difference represents a colorimeter reading of only 0.005 and is therefore not significant. In another patient (A.W.) a similar experiment yielded the results 152 and 164 for serum and plasma respectively, representing a colorimeter difference of 0.014, still within

experimental limits.

Having thus determined that comparable results can be obtained from plasma and serum, and because it is easier to obtain more plasma than serum from the same amount of blood, it was decided to do all estimations on heparinized blood - 1 mgm. of heparin to 7.5 ml. of venous blood.

Where possible, all specimens were collected from the patient with a dry syringe and in the fasting state or 4 - 6 hours after the last meal (generally just before lunch). The blood was centrifuged immediately and if not assayed the same day, stored frozen solid at - 10° C. Deterioration of peptic activity does not occur with storage of 3 weeks at - 10°C.

Plasma from J.N. 24.5.51. Colorimeter score .185; on 15.6.51,.170. (=31.2 P.U.% cf.32.7 P.U.%)

Another plasma assayed on 27.4.51 gave the result 140 P.U.% and on 18.3.52 (10½ months later) 57 P.U.%, a loss of about 55% of the original enzyme, and on 9.2.53 was 43.5 P.U.%, a loss in 2 years of 68% of original value.

It appears safer, therefore, to assay serum or plasma, if stored at - 10°C. within 3 weeks.

Gastric juice, on the other hand, probably cannot reliably be stored for any length of time, because the pH of the juice is often at the acid level where autodigestion of pepsin is likely to occur (Northrop, Kunitz and Herriot 1948).

UNITS.

Expression of results in the haemoglobin method.

In deciding on the adoption of the haemoglobin method of peptic assay mainly because of reproducibility of results with different samples of substrates, most authors have failed to reach agreement on the exact definition of arbitrary units, even if the definition thereby has to be arbitrary.

It is agreed that the determination of concentration alone is not accurate enough for comparative studies and the use of this method has been dropped in favour of the rate of excretion except by Farnsworth, Speer and Alt (1946). Bucher (1947) expresses results as mgm. tyrosine per 24 hours, determined from a 60 minute digestion of 5 cc. 4% Hb (0.2 G. Hb.) by 1 cc. of test fluid. The normal range is 35 to 693 mgm/24 hours. Mirsky et al define a unit as that amount of enzyme which will release 1 mgm. of acid soluble tyrosine-like material in the standard 10 minute assay (of 5 cc. 2% Hb. by 1 cc of urine at 37°C.) Later they incubated for 30 minutes but then divided by 3 to give 10 minute units. These are expressed as log units per hour, i.e. a rate of excretion. Gray et al (1950) define one unit as that amount of enzyme releasing 0.4 mg. tyrosine-like substances at 37°C. in a 30 minute incubation from 0.1 G. Hb. substrate, and express their results as mg. tyrosine/24 hours. Janowitz, Levy and Hollander (1950) define a unit as that amount of enzyme which releases 1×10^{-4} mEq. of tyrosine from a standard 10 minute assay, and expressed as units per hour. These units will be designated by the abbreviated symbol P.U. for $P.U._{10}^{Hb}$ meaning peptic units in a 10 minute incubation of haemoglobin under standard conditions.

The latter definition has been adopted in this study (Hirschowitz 1955) for reasons already given, viz. the practicability of a 10 minute incubation period, the economy of using 0.1 G. Hb. instead of .2 G. as Bucher (1947) does, and because the amount of colour produced is within the range of the standard, viz. 0.0181 mg. or 1 mEq. per ml., and finally that the use of mEq. is more elegant in chemistry than the metric system.

To convert results of longer digestion periods and other methods to standard units, it is necessary to reduce the digestion time used to common denominator of a 10 minute period by simple division, since results are arithmetically linear, and to convert mgm. to mEq. dividing by 181 (mol. wt. of l-tyrosine). It is finally necessary to reduce the time of the excretion as given to 1 hour, since it is absolute that 1 hour is an accurate part of say a three or twelve hour assay but not entirely justifiable to assume that a 5 hour assay is an accurate part of the other 21 hours of a 24 hour period. One hour should therefore be used as a common denominator in expressing results as a rate of excretion. If full 24 hour collections of urines are assayed, then, of course, the expression per 24 hours is as justified as per hour, though for universal comparison the hourly rate of excretion would be more applicable.

Plasma pepsinogen results to conform to urine units by definition should be reduced to the equivalent 10 minute units by division by 6, since incubation is for 60 minutes. It has been shown (p. 62) that the results are proportional to time of incubation. However,

plasma pepsinogen must be expressed as a concentration and to conform to other estimations in clinical biochemistry, results are given as 10 minute units per 100 ml. of serum or plasma

CHAPTER IV

THE PHYSIOLOGY OF PEPSINOGEN

This chapter deals with the secretion of pepsinogen by the chief cells of the stomach into the gastric lumen where it is transformed into and measured as pepsin; it deals with the diffusion of pepsinogen directly into the blood from the peptic cells; the diffusion through the body; the mechanism of excretion by the kidney not previously described; and the influence of physiological variations on the amount of pepsinogen in the urine.

SECTION I

It would perhaps be best to describe briefly the present state of knowledge upon which this study was built. It is almost 120 years since Schwann (1834) postulated the existence of pepsin. In 1881 Langley published his discovery of pepsinogen and the observation of the secretory cycle of the pro-enzyme granules in the chief cells of the stomach. He showed that pepsinogen was converted to pepsin in the stomach and destroyed in the small intestine. The existence of "pepsin" in the urine had by then been known for 20 years (Brücke 1861) and it had been assumed that the enzyme was pepsin. The work of Langley led to speculation as to the exact nature of the urinary enzyme, and by inference Gehrig (1886) assumed that it was pepsinogen and that it was absorbed directly by the blood from the gastric glands. However, as late as 1910 Ellinger and Scholz from crude inactivation studies still believed the urinary enzyme to be a mixture of pepsin and pepsinogen.

It was later shown by Gottlieb (1924) that it was pepsinogen, but the final proof as to its nature and origin was the composite of many papers culminating in the excellent work of Mirsky and his co-workers (1948) and of Janowitz et al (1950).

Its origin in the stomach was shown by the work of Frouin (1904) in the dog, who found that removal of the stomach or of its mucous membrane resulted in an apeptic urine, but that exteriorization of the stomach so that all its secretions were immediately drained out, did not cause the disappearance of urinary pepsinogen. This work was later repeated and confirmed by Bucher and Ivy (1947) in cats and in rats by Balfour, Preston and Bollman (1948) and in the isolated human case in several papers (Janowitz, Levy and Hollander 1950; Podore et al 1948). Collateral evidence came from the near-absence of pepsinogen found in the urine by almost every author on the subject of achylia and pernicious anaemia (see Farnsworth, Speer and Alt 1946 and Chapter V.) The one dissenting voice in all this work was that of Strauss (1926) who found that there was always some secretion of pepsinogen in the urine and postulated a "parasekretion" - a source of enzyme other than the stomach.

The nature of the urinary enzyme has not been proved conclusively, i.e. it has not been isolated and shown to be pepsinogen by physico-chemical methods. However, the work of Mirsky et al (1948) is very strong evidence in favour of the identity of the urinary pepsin-like enzyme with pepsinogen. This confirms the modern views derived from

inactivation studies on the urinary enzyme. Mirsky et al showed that oral feeding of pepsin or pepsinogen produced no rise of urinary enzyme; also that intravenous pepsinogen did produce a rise in urinary enzyme indistinguishable in its behaviour from pepsinogen. It had been shown before by Voight (1923) that intravenous or subcutaneous pepsin produced no rise in urinary pepsin and she correctly interpreted this as the result of destruction of the enzyme at the pH of the blood.

The knowledge of how pepsinogen behaved in its course from the moment of absorption from the gastric secretory tissue until it appeared in the urine was very limited. Benderskey (1890) showed that pepsinogen appeared in the sweat; Loeper and de Bray (1921) assayed pepsinogen in the blood, but believed it was pepsin. Gottlieb (1924) estimated the blood enzyme, found it to be stable from day to day and found, as Loeper and de Bray (1922) had, that it rose in uraemia. Since this work was done, Mirsky et al (1952) have published a paper dealing with blood pepsinogen in disease and have reached essentially the same conclusions as I have. Their paper on the source and properties of the blood enzyme is interesting and comprehensive. (see p.122 for discussion.)

Little, then, was known about the mode of trnsport of pepsinogen in the blood, and the statements of van Calcar (1910) that it was carried in the leucocytes was as unproved as Gottlieb's contention that it was carried as free pepsinogen in the plasma, although the latter had been more widely accepted and I believe it to be

substantially true.

About the mechanism of excretion by the kidney nothing was known except that excretion was interfered with by chronic nephritis; van Noorden (1893) claimed that it was increased, Strauss (1926) and Rothschild (1930) that it was decreased. Bucher (1947) believed that pepsinogen was simply excreted as were other waste products and Mirsky (1950) believed that none was reabsorbed in the tubules.

Conflicting views are current as to the relation between urinary pepsinogen and gastric secretory activity. The most convincing work on the subject by Janowitz and Hollander (1951) showed an almost mathematical relationship; that of Varro et al (1952) almost no relationship. Gottlieb (1924) showed that there was some relation between urinary pepsinogen and gastric secretion. He was unable to show a relation between gastric hydrochloric acid and urinary pepsinogen but showed that there was a positive correlation with the volume of gastric secretion.

The evidence of Bucher and Anderson (1948) who showed that stimulation of gastric secretion by caffein and by histamine had no effect on urinary pepsinogen in cats and the observations of Mirsky et al (1948) showing the lack of effect of a wide range of factors, including food, sleep and activity throws some doubt on the results and conclusions of Janowitz and Hollander (1951). Evidence to be presented here supports that suspicion.

The evidence on the control of gastric and urinary pepsinogen by the adrenal hormone Cortisone and by A.C.T.H. has all been presented by one group of observers - Spiro et al (1950) in four different papers. Evidence to be presented suggests that their results might be inaccurate and their conclusions biased.

The facts then that were known when this investigation was started in 1951 were that pepsinogen, the inactive precursor of pepsin, was formed only in the peptic cells of the stomach; from there most was secreted into the stomach and there autocatalytically changed to pepsin which was then destroyed in the small bowel and not reabsorbed. Some of the pepsinogen diffused directly into the blood from the peptic cells and was transported as free pepsinogen in the plasma to the kidney where it was excreted freely "as other waste products" (Bucher 1947). There was conflicting evidence on the relation of urine pepsinogen to gastric secretory activity and it had been suggested that the adrenal influenced the secretion of gastric pepsinogen.

RESULTS.

Experimental evidence to be presented in this chapter deals with all the aspects of the physiology of pepsinogen:-

(a) Firstly the secretion of pepsin in the stomach has been investigated using overnight 12 hour basic secretion tests, both qualitatively and quantitatively in relation to other gastric constituents and also in relation to plasma and urine pepsinogen. The effects of caffein, histamine and adrenal steroid hormones are shown.

(b) The next section deals with blood pepsinogen and concerns an original method for its estimation which has made possible its identification, the proof of its sources in the stomach and in the leucocytes, and its distribution throughout the body fluids. Normal variations as well as the effects of disease are discussed. Antipepsins are shown to be present in pus, but absent from the blood.

(c) The next section deals with a new concept of renal excretion of pepsinogen, made possible by the development of the new technique for plasma pepsinogen estimations. This is based on simultaneous clearance studies done of creatinine and pepsinogen which proves that pepsinogen is filtered through the glomeruli and reabsorbed in the tubules, and is in keeping with the modern views of protein filtration and reabsorption in the kidneys.

(d) Observations are then presented on pepsinogen in the urine - its constancy in the individual, its relation to volume, to chloride excretion and diurnal variations. These observations confirm most previous work. Evidence is presented to show that some pepsinogen

in the urine arises from that formed endogenously in the leucocytes.

(e) The effects of operations, both gastric and non-gastric, are shown on a number of cases, and interpreted partly in relation to the concept of renal filtration and reabsorption.

(f) The effects of the adrenal have been studied on pepsinogen metabolism, comprising observations of the effect of pituitary-adrenal deficiency diseases as well as experimental observations on the effect of A.C.T.H. and cortisone. These results contradict earlier authors.

SECTION II

Gastric Secretions.

Gastric secretion of pepsin was not studied in great detail, since so much has already been written about the subject.(Babkin 1950). However, some studies were undertaken with the following objects:

(1) To determine the hour to hour variations in fasting, sleeping subjects in relation to the pH, acid and volume of secretion. 11 test meals were performed on 8 subjects.

(2) To determine the relation between pepsin and other constituents of gastric juice, and pepsinogen of urine and plasma - 12 such experiments were done on 10 subjects.

(3) To determine the effect of histamine on pepsin secretion in relation to acid and volume of gastric juice - there were 3 experiments in this group.

(4) To determine the effect of caffein by mouth on gastric pepsin and simultaneously on plasma pepsinogen - 6 such experiments were performed.

(5) To determine the effect of A.C.T.H. and cortisone on gastric secretion in one patient.

Technique

The tests were performed with the patient in bed. A Levine tube with a metal piece included in the tip was passed at 4 p.m. and the patient screened to determine the position of the tip of the tube, which was adjusted to the angulus. Small samples were withdrawn at hourly intervals from 5 p.m. to 8 p.m. and the juice tested for free

acid and the pH determined to avoid doing the estimation on achlorhydric patients. The patient was allowed a light dinner at 6 p.m. but nothing more by mouth until the test was complete the next morning.

At 8 p.m. the stomach was emptied as completely as possible and a suction pump applied to the tube. The complete contents of the container of the suction pump were emptied into a labelled container on the hour until completion of the test at 8 a.m. the next morning. A simultaneous 12 hourly specimen of urine was collected. The position of the tube-tip was again checked on X-ray and the test discarded if not at the angulus.

The following observations were then made on each specimen of gastric juice - volume, pH (before and after filtering of the mucus) and on the filtered juice. (See Methods, ChapterIII). The free and total hydrochloric acid was measured with Topfer's indicator and the pepsin concentration determined by the standard haemoglobin method. The hourly secretion rate of pepsin and acid, as well as concentrations of these substances were calculated.

Of the 11 12-hour test meals done on 8 subjects, there appeared to be two in whom the pepsin concentration varied almost directly with the pH of the gastric juice and not with the hydrochloric acid. These were subjects in whom there was very little free acid and who were often achlorhydric. One typical

such graph is shown below.

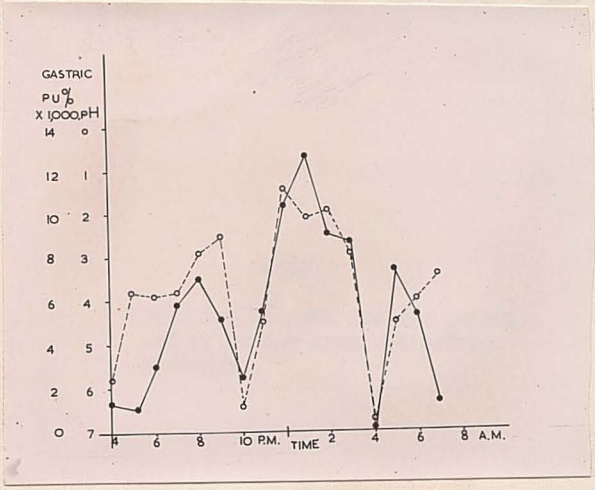
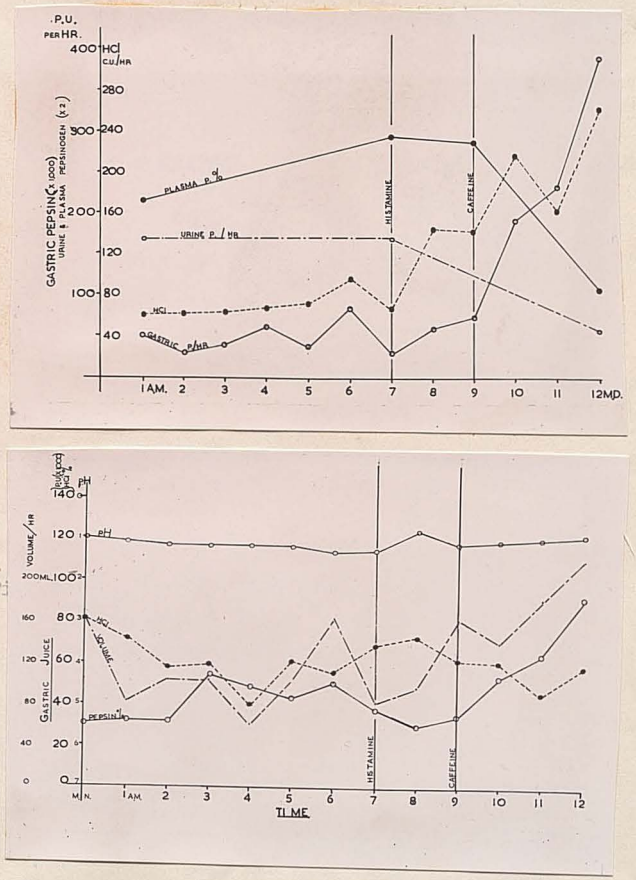


Fig. 9. Gastric pepsin secretion Type I.
Relation between gastric pepsin and pH in hypochlorhydric patient.

On the other hand, there were three patients in whom no relation at all existed between gastric pepsin and pH of gastric juice, but in whom there was a close relation between gastric pepsin and gastric hydrochloric acid when both were measured as a rate of secretion per hour but not as concentration. This difference is compared in the two graphs (a) and (b) shown below.



Figs. 10a. and b. Gastric pepsin secretion Type II. Relation between gastric pepsin and HCl when measured as rate of secretion (a) but not as concentration (b).

A third type of patient (there were 3 in this group) showed no relation at all between pepsin secretion and either gastric juice volume, pH or acid.

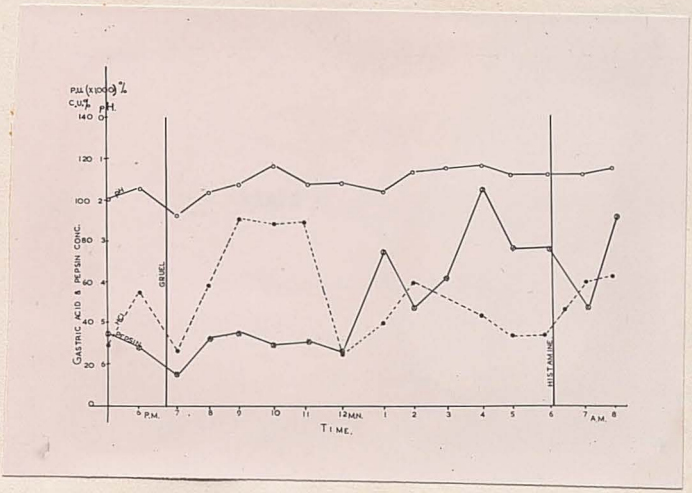


Fig. 11. Gastric pepsin secretion Type III. Lack of relation between pepsin and other constituents of gastric juice.

The test is an uncertain one for many reasons - mainly because of the uncertainty as to whether complete emptying of the stomach is ever obtained; because of unpredictable emptying of the stomach into the duodenum and because of duodenal regurgitation into the stomach. Because the estimation of pH, acid and pepsin and the calculations involved are tedious (the estimations on one such test meal take the best part of a day), it was felt that the results did not justify the labour and material and discomfort to the patient.

THE RELATION BETWEEN GASTRIC PEPSINOGEN AND OTHER CONSTITUENTS AND THE PEPSINOGEN IN THE URINE AND PLASMA

As will be shown in Chapter V, there is an overall similarity in the gastric HCl and urinary pepsinogen in duodenal ulcers. Janowitz and Hollander (1951) attempted to show more clearly this correlation by estimating the pepsinogen content of simultaneously obtained gastric juice and urine over a 3 hour period and prove their contention that there is a close relation between the pepsinogen content of these fluids. (see p. 174).

Results.

Experiments were conducted along two lines - in five patients simultaneous gastric juice and urine were obtained over a 3-hour period as done by Janowitz and Hollander (1951) in the fasting state and a sample of blood was taken at the end of this test.

In 8 other patients, 11 twelve-hour test meals in the fasting

state were performed as described above and simultaneous urine collections were made. Blood samples were taken in the fasting state.

In four other patients, only simultaneous blood specimens were taken with one basal specimen of gastric juice.

The following table summarizes all these findings.

(see over for table)

TABLE NO: 11 RELATION BETWEEN GASTRIC PEPSIN, & ACID AND PLASMA AND URINE PEPSINOGEN.

Name	Duration of test. (Hrs.)	Gastric Pepsin % P.U.%	Gastric Pepsin/hr P.U./hr.	Mean HCl cc N/10%	Plasma Pepsinogen % P.U.%	Urine Pepsinogen/hr. P.U./hr.
A.Sa	15	5,360	-	0	0	0
T.Hp	12	63,250	52,100	-	32	282
W.H.	13	64,200	44,400	93	51	240
A.Br	11	79,450	-	4.5	-	500
F.Do	13	42,800	12,580	51	-	405
J.St	10	65,600	69,950	62.5	84.5	335
C.Bo	10	50,320	25,290	8.7	28	76
W.Tu (1)	12	118,000	71,400	27.3	53	190
(2)	12	121,000	115,500	15	31	260
(3)	12	85,000	88,300	44	28	300
(4)	12	104,000	75,670	38	51	420
B.Ba	3	15,600	1,120	-	17.5	27
B.Jo	3	57,000	54,000	0	29	47
C.Ne	3	84,800	65,000	30	25.5	85
H.Br	3	124,000	108,000	34	18.7	185
C.Bo	3	9,500	4,560	0	10.5	108

TABLE NO: 11. (Continued)

D.O.	-	176,000	-	-	59	-
C.O	-	285,000	-	-	64	-
M.O	-	86,000	-	-	14	-
F.A.	-	18,300	-	-	14	-

If any correlation does exist, it does not do so between plasma and urine pepsinogen per se, unless urine flow be taken into account, i.e. unless renal clearances are calculated and this has been shown to be a rather haphazard relation (see renal excretion of pepsinogen, Chapter IV).

Correlations were therefore attempted to show if there were any relationship between gastric pepsin and either urinary pepsinogen or plasma pepsinogen.

The first graph below shows the concentration of gastric pepsin in P.U.% plotted against plasma pepsinogen in 18 cases (These excluded any cases in which the gastric secretion was stimulated either with caffein or histamine and are all basal secretions. Superimposed on the graph is a line drawn from 0 0 through the point where the mean values of gastric pepsin and plasma pepsinogens intersect (the theoretical mean). It would appear from this graph that some linear relationship exists between gastric pepsin concentration and plasma pepsinogen concentration. If this is so it is only valid for the stomach in the resting phase or in a state of basal secretion, since the stimulation of pepsin secretion by caffein or histamine (see below) causes a fall in plasma pepsinogen. However, 18 instances are not enough to give a clear picture of the relationship.

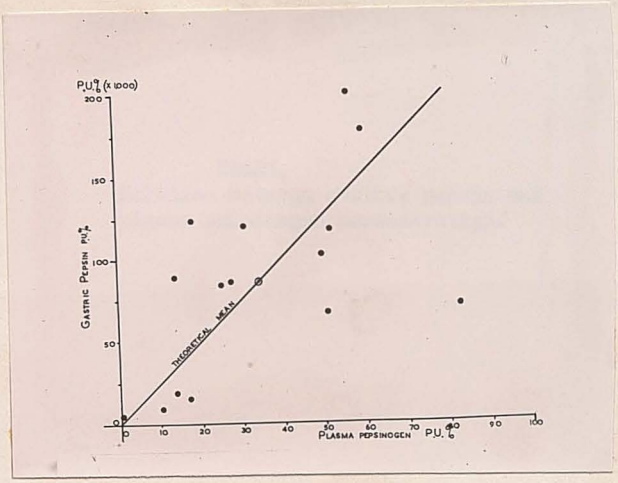


FIG. 12. RELATION BETWEEN GASTRIC PEPSIN AND PLASMA PEPSINOGEN CONCENTRATION.

The next graph drawn up from the foregoing table shows the attempt to correlate gastric pepsin secretion with urinary pepsinogen excretion estimated on simultaneously obtained specimens.

In this graph, 17 experiments are shown and both concentrations and rate of pepsin secretion are plotted against rate of urinary pepsinogen excretion in P.U. per hour. The

result is shows no correlation ge ve, either for the concentration of pepsin or for the rate of secretion per hour of gastric pepsin. Once again it should be stressed that the small numbers involved make conclusions hazardous.

(see fuller discussion p. 172).

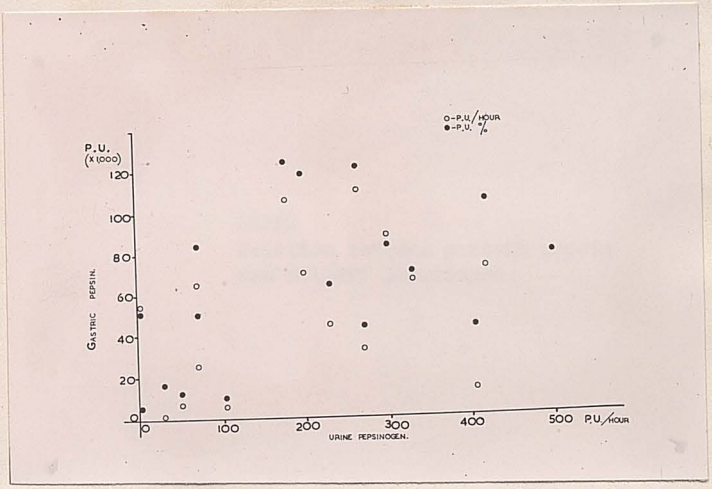


Fig. 13. Scatter diagram showing lack of correlation between gastric pepsin and urinary pepsinogen

By simple inspection of the relation between the mean gastric hydrochloric acid and any of the other estimations, it will be seen that there could be no possible correlation, either with gastric pepsin or with pepsinogen in the plasma or gastric juice.

The effect of histamine on gastric pepsin

The effect of histamine was studied on 3 patients who had 12-hour test meals preceding in order to be certain of the patients' basal level of secretion. The technique was as described above i.e. continuous gastric suction in the fasting state with measurement of the gastric juice on the hour. After histamine complete ½ hourly specimens were taken for 2 hours. The pepsin was calculated both as concentration in each ½ hourly specimen and as total amount excreted per hour. In case 3 there was emptying of the stomach with regurgitation of duodenal contents which invalidates the second hour of this test. The first two patients had duodenal ulcers and the third a gastric ulcer. The following table sets out the results of these three experiments.

TABLE NO:12 EFFECT OF HISTAMINE ON GASTRIC PEPSIN AND ACID

	Name	Mean basal level	½ hour	1 hour	1½ hours	2 hours after histamine.
Pepsin Conc. P.U.%	F.Do	42,800	43,000	52,800	68,000	116,000
	J.St	65,600	45,000	49,500	37,600	68,000
	C.Bo	50,320	101,000	133,000	149,000	7,300 (bile regurg.)
P.U. per hr. Rate of secretion	F.Do	12,580		2,460		12,850
	J.St	69,950		88,000		93,000
	C.Bo	25,290		54,600		13,100 (bile)
HCl ccN/10 %	F.Do	51	58	62	64	62
	J.St	62.5	71	75	66	58
	C.Bo	8.7	13	20	15	0 (bile)
Mean Values for 3 patients	P.U.%	52,910	63,000	87,900	83,870	92,000 (2 only)
	P.U./Hr.	35,940		48,350		57,925 (2 only)
	HCl%	40.7	47.3	52.3	48	60 (2 only)

From these figures it will be seen that histamine causes not only an increase in acid (and volume of secretions) but an increase of pepsin both in concentration and in total amount.

These results deny the statements that histamine causes an increase of gastric pepsin merely by washing out pepsin from the cells (Babkin 1950) - the pepsin keeps on increasing both in concentration and in total amount up to two hours. As will be shown in the next section, caffein given after histamine causes a still further increase in the secretion of pepsin up to 5 hours after the start of the test.

The action of caffein on gastric pepsin and blood pepsinogen

Seven experiments were performed to test the effect of caffein on gastric secretion of pepsin. In all these patients simultaneous plasma pepsinogen concentrations were estimated, in order to try and elucidate the problem of whether the secretion of pepsinogen in response to caffein was an active or a passive event.

Technique: In four subjects (2 duodenal ulcers and 2 controls) one fasting specimen and one 70 minutes after caffein were taken.

In three subjects caffein was given 2½ hours after 0.5 mgm. histamine and the patients observed for a further 3 hours. These patients had had 12 hours previous intubation in the fasting state to determine the basal secretion pattern. The caffein was given as a draught of 100 Gm. of caffein citrate in 200 ml. of water, no suction being applied for ½ hour, the stomach then being emptied. Then continuous suction was applied, and complete

½ hourly specimens of gastric juice obtained and these were assayed for pepsin and titrated for acid. The pH was also measured on these. Blood samples were taken at the time of administration of the caffein and 3 hours later.

The results of the first four subjects are as follows:

TABLE NO: 13 ACTION OF CAFFEIN ON GASTRIC AND PLASMA PEPSINOGEN

	FASTING LEVELS		70 MINUTES AFTER CAFFEIN	
	Gastric Pepsin P.U.%	Plasma P.U.%	Gastric pepsin P.U.%	Plasma P.U.%
D.O	176,000	59.3	112,000	62
C.U	285,000	62	213,000	75.3
F.A	8,600	14	10,900	14
M.O	18,300	14	19,000	15.8

The three other patients were studied in greater detail as the following table shows and the conclusions to be drawn from these figures are probably more valid.

The table shows the mean values for the periods studied which were consecutive and all in the fasting state - 12 hour basal s cretion (sleeping) 2 hours histamine followed by caffein and a wait of ½ hour, and then 3 hours observation.

TABLE NO: 14 THE EFFECT OF HISTAMINE FOLLOWED BY CAFFEIN ON GASTRIC SECRETION; AND PLASMA AND URINE PEPSINOGEN.

Pt.	GASTRIC JUICE Vol. ml./hr.	pH	P.U.%	P.U./Hr.	HCL ccN/10%	HCL ccN/10/Hr	PLASMA * P.U.%	CSC /	URINE P.U./Hr.	Vol. ml./hr.
J.St.										
Basal	109	-	65,600	69,950	62.5	70	84.5	8.2	335	36.7
Histamine	128	-	50,000	90,500	65	136	78.3	)	) 114	
Caffein	193	-	75,800	269,000	57	212	35	6.4	)	12.8
C.Bo										
Basal	33.6	-	50,320	25,290	8.7	2.2	28	11.1	76	100
Histamine	29	-	97,500	34,000	16	4.1	42	)		
Caffein	34	-	148,000	51,000	10	4.5	26	20.3)	310	43
F.Ho										
Basal	77	2.26	-	-	-	-	109	4.1	475	41.7
Histamine	59	-	-	-	-	-	102	)		
Caffein	107	2.47	-	-	-	-	83.5	10.5)	475	57

* Plasma levels were estimated before and after basal secretion, 2 hours after histamine and 2½ hours after caffein.

/ CSC - Renal clearance of pepsinogen in cc/min.

Conclusions

In drawing conclusions, the single observations on the first four subjects must be interpreted cautiously, since no allowances were made for dilution and it is not certain whether the peak of secretion was measured.

In the second set of observations it is not certain whether the histamine potentiated the action of caffein, which it appears to have done. The reverse is known to be true (Babkin, 1950) Nevertheless, the figures show conclusively that caffein after histamine produces a great rise in pepsin secretion both in concentration and in absolute amounts; this is true for acid secretion as well. The continued increase of pepsin in the gastric juice after 17 hours fasting and after 5 hours of stimulation of gastric secretion, first by histamine and then by caffein, would suggest that the secretion of pepsinogen by the peptic cells in response to stimulants is an active process, rather than a simple washing out phenomenon as suggested by Babkin (1950). It further suggests that pepsinogen is formed continuously, and probably fairly rapidly, since the amounts secreted exceed the probable amounts that could be stored preformed in the gastric mucosa.

The decrease in plasma pepsinogen which occurs simultaneously with the great increase in gastric pepsin secretion is interesting. It suggests firstly that the diffusion of pepsinogen into the blood is a continuous event; it shows that no pepsinogen is reabsorbed from the gastric lumen, since if it were there would

be an increase with the increase in gastric pepsin; it suggests that pepsinogen diffuses into the blood from the bases of the peptic cells because mobilization and discharge of pepsinogen in these cells occurs from the base to the free end of the cell(Gillespie 1898). It does not assist in deciding whether the process of secretion in response to stimulation is an active or a passive process.

It is just possible that the blood could act as a reservoir for pepsinogen of the peptic cells.

The simultaneous estimation of urinary pepsinogen shows again the lack of obvious relationship between gastric pepsin and urinary pepsinogen. It is certain, however, that the relation could not be a simple one. Since the complex factor of renal adjustments to the dehydration involved in starvation coupled with removal of large volumes of gastric juice (approximately 2,000 ml., 600 ml. and 1500 ml. in the above three cases respectively) can only be guessed at from the figures shown above, we must assume that any attempt to relate gastric pepsin and urinary pepsinogen, which does not take the active role of the kidney into account, is inaccurate and misleading.

The renal clearances show that where the urine pepsinogen fell, there was a fall in the clearance and vice versa to account for the changes. These figures without simultaneous glomerular filtration rate estimations only suggest, but do not prove, that there is a change in reabsorption of this protein, not dependent

on the source of production, the state of hydration or blood levels alone.

The effect of A.C.T.H. and of cortisone on gastric secretion

This aspect was studied in one patient, W.Tu. a 49 year old male with a duodenal ulcer of 5 years duration.

Four overnight 12 hour test meals were performed, the first before, the second immediately following 50 mg. A.C.T.H. intramuscularly, the third 5 days later and 2 days after 200 mgm. Cortisone I.M.I and the fourth 4 days after cortisone.

The following table summarizes the information so obtained on the gastric juice, with simultaneous blood and urine estimations.

TABLE NO: 15 THE EFFECT OF A.C.T.H. AND CORTISONE ON GASTRIC SECRETION.

	Volume ml./hr.	HCL N/10 cc. Mean Conc. %	Per hour	pH mean	Pepsin Mean PU %	Mean P.U./Hr	Plasma P.U.%	Urine P.U./Hr.
Basal	69.5	48.5	27.3	1.58	118,000	71,400	53	190
With A.C.T.H.	90	39.2	15	2.15	121,000	115,500	31	260
2 days after Cortisone	109	52.5	46.8	2.11	85,000	88,500	28	300
4 days after Cortisone	66.6	62.0	38.25	2.01	104,000	75,670	51	420

These results would seem to indicate some slight increase in gastric juice volume and acid, but little effect on pepsin in the gastric juice. The increase in the urinary pepsinggen is due to an increased renal clearance as shown elsewhere (p. 104) and is probably due to a direct effect of the hormones on renal mechanisms affecting pepsinogen excretion.

II. PEPSINOGEN IN THE BLOOD

There are only four references in the available literature relating to the study of pepsinogen in the blood (Saxl 1915; Loeper and de Bray 1922; Gottlieb 1924; and Mirsky et al 1952)

All other references only touch indirectly on blood pepsinogen and are concerned, mainly theoretically, on how pepsinogen is transported from the stomach to the urine.

The first theory was that pepsin was reabsorbed from the gut lumen into the blood. (Brücke 1861). Shortly after that Gehrig (1886) stated that none was reabsorbed but that it diffuesd directly into the blood stream from the peptic cells, following on the work of Langley (1881) who showed that pepsin was destroyed in the gut lumen and that it arose from the peptic cells from a precursor which he called pepsinogen.

The theories as to its physico-chemical state in the blood eventually gave way to the theory of its existence as free pepsinogen in the serum. The earlier theories are worth listing briefly.

(1) Adsorbed onto fibrin: Leo 1885 and Stadelman 1888 believed that "pepsin" was adsorbed onto fibrin and this formed the basis for the fibrin method of pepsin assay used for some years. Fuld and Hirayama (1912) disclaimed the idea by showing that at optimum acid pH fibrin showed no evidence of digestion which could be attributed to adsorbed pepsin. Gottlieb, however, in 1924, in

experiments rather more accurate than those of his predecessors, believed that some adsorption onto fibrin occurs, but only at acid pH and that normally pepsinogen circulates as such in the plasma.

(2) In the white blood cells: Von Calcar (1910) believed that pepsinogen was transported as such in the leucocytes and claimed that it disappeared after total gastrectomy. He gives no other results nor any figures and one must assume that he found no pepsinogen in the plasma.

(3) Van Noorden (1893) believed that pepsin circulated combined with an inhibitor complex and Fuld and Hirayama (1912) demonstrated to their satisfaction antipepsins - pepsin complexes. Peczenik and Kawahara (1927) fully supported and confirmed these statements and believed that this complex was set free in the kidney. The presence of antipepsins in the blood was denied by Hamburger (1911) and by Oppenheimer (1939) and as will be seen later, I was unable to demonstrate any antipepsin in the blood. Furthermore, pepsinogen can be shown to be present in the free state in the blood.

(4) Free pepsinogen in the plasma, then, is the only alternative left, since pepsin per se is denatured at the pH of blood (Voight 1923). The view that free pepsinogen is the method of transport in the blood was supported naturally by the authors who found free pepsinogen in the plasma.

A brief report from Paul Saxl (1915) mentions only that

pepsinogen was detected in the serum and quotes a few figures to show reproducibility of results. 20 normals all had serum pepsinogen. Loeper and De Bray (1922) invented an ingenious technique - they simply acidulated serum with HCl and incubated for 22 hours, estimating the difference in albumin content between incubated and control sera. They found that blood pepsin is stable for each subject in the fasting state and elevated after a meal, and in a dog with the ureters tied, and in chronic nephritis, and also, without giving supporting figures, concluded that it varied with gastric activity and paralleled urinary pepsinogen. While their method is ingenious, one feels that the method for albumin estimation on which it is based is not accurate enough to have allowed them to draw such detailed conclusions.

Gottlieb (1924) investigated blood pepsinogen fairly fully, using a more reliable method (Edestin; see Chapter II) and found that serum content was the same as plasma content. He incubated his specimens for 120 hours at 40°C. He compared his results with standard digestion mixtures of Armour scale pepsin and found that the blood pepsinogen varied from 1.5 to 2.1 γ pepsin equivalent per ml. of serum in normals. He grouped his subjects according to gastric acidity - in 68 controls with normal acid, serum pepsinogen varied from 1.0 - 4.0 γ per ml. (mean 2.0); in 25 hyposecretors from 1.2 to 1.8 γ per ml. (mean 1.4) and in 18 hypersecretors 1.8 to 4.8 (mean 2.5); in 2 nephritics levels of 4.0 and 4.8 γ were found. In pernicious anaemia he found plasma pepsinogen about 1/100th normal. He found that there was

little daily variation except in dogs who showed an increase 2 to 3 hours after a high protein feed; and a gradual decrease with starvation. Renal clearances which I calculated from Gottlieb's figures on 3 patients are 16.5, 18 and 19, and 14.2 cc. per minute. respectively. These mean that Gottlieb's figures are very near to those found in this study by a different method. The main objection to Gottlieb's method is the need to incubate the digest for 5 days (See Methods, ChapterIII).

Following Gottlieb's (1924) publication no mention seems to have been made of blood pepsinogen or any proteolytic enzyme active at very acid pH. Barnes (1940) demonstrated all the proteolytic enzymes in the white cells, using the haemoglobin method, but makes no mention of any proteolysis below pH 3.5. From pH 3.5 to 4.0, cathepsins caused slight proteolysis

Results.

At the time this study was undertaken no advances had been made on Gottlieb's (1924) work. It was felt that if there was to be any advance in the knowledge of the physiology of pepsinogen a simple and reliable method was necessary for the determination of blood pepsinogen. In the search for such a method, no refinement was found possible of Gottlieb's (1924) method and this was discarded as being too cumbersome and not certainly accurate. After many trials a method which was simple, quick and reliable was found.

This method enabled me to confirm the existence of such a proteolytic enzyme; partly to identify the enzyme as pepsinogen, to determine its sources and show its distribution in the blood and other body fluids. Its normal and pathological variations were determined and it was this method which made possible the discovery of the mode of excretion of pepsinogen by the kidneys.

(i) The existence of proteolytic enzyme in the blood.

The preliminary experiments, after a satisfactory method had been obtained, were designed to show the proteolytic activity of blood at varying pH.

Method:

0.2 G. of haemoglobin substrate was mixed with 2.18 ml. of plasma, the pH adjusted with 0.05 N. HCl to the desired level (using the Cambridge pH meter) and the volume made up to 12.5 ml. Of this, 6 ml. was precipitated immediately with 5 ml. of 10% trichloracetic acid and 6 ml. incubated for 1 hour at 37°C and thenprecipitated. The difference in tyrosine content of the filtrate is determined and peptic activity determined by the usual method. The same method was employed for whole blood of the same patient. Six experiments were performed on each plasma and whole blood with pH ranging by unit from 1 to 6.

Results:

The following table and graph illustrate the results.

PLASMA		WHOLE BLOOD		DIFFERENCE BETWEEN WHOLE BLOOD AND PLASMA
pH	P.U.%	pH	P.U.%	+ or -
1.01	192	1.0	353	+ 161
2.01	288	2.0	242	- 46
3.02	545	3.0	410	- 145
4.0	198	4.0	340	+ 142
5.0	96	4.95	360	+ 264
6.1	288	5.94	236	- 52

TABLE NO. 16. PROTEOLYTIC ACTIVITY OF BLOOD AND PLASMA AT pH 1 - 6

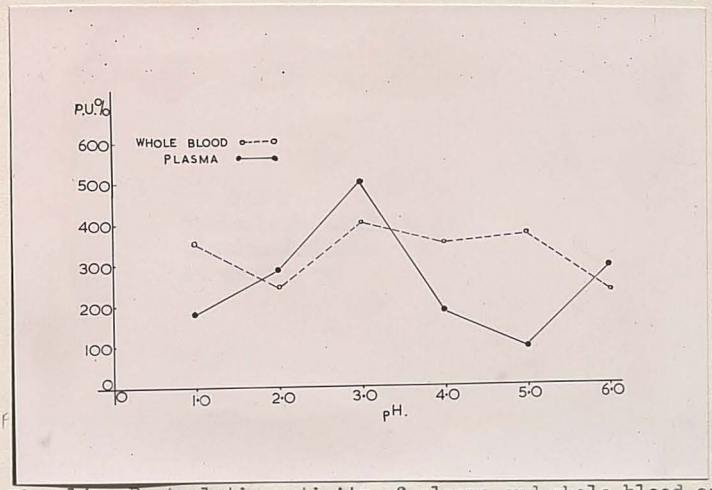


Fig. 14. Proteolytic activity of plasma and whole blood at pH 1 - 6

Conclusions

These results indicate that both in whole blood and in plasma there is a proteolytic enzyme capable of digesting haemoglobin at pH 1 - 3, as well as other active proteolytic enzymes up to the end of the range of pH tested (pH 6.0). From the specificity of enzyme pH optimum one must assume that the enzyme acting at pH less than 3.0 is pepsin, unless it be an as yet unidentified enzyme. Since we know that at pH over 7.0 pepsin itself is denatured and the pH of the blood is over 7.0, and if we assume that this enzyme is pepsin, then it must exist in the blood as the inactive precursor of pepsinogen. The inability to demonstrate any antipepsin means that the proenzyme is probably in the free state.

The proteolytic activity exhibited at pH greater than 3 and up to pH 4.5 is probably partly due to pepsin; that from pH 5.5 to 5 probably catheptic and over 5.5 probably tryptic (Merten and Ratzer 1949).

The differences shown between the proteolytic activity of plasma and of whole blood indicate that there is probably not an equal distribution of proteolytic enzymes between plasma and cells.

(ii) The identity of the proteolytic enzyme of the plasma active at pH 1 - 3.

While the theoretical arguments in favour of the proteolytic enzyme of plasma active at pH 1 - 3 being pepsinogen are very strong

(see above) there is no certainty that it is in fact pepsinogen.

Since, as in urine, the enzyme content of blood is so small that it could not possibly be extracted and identified chemically, one has to attempt indirect methods of identification.

Results

Firstly it was shown (see p. 123) that the enzyme is present in greater concentration in gastric vein blood than in a peripheral vein. This suggests that it arises from the stomach.

Secondly, attempts were made to identify the enzyme by inactivation studies, both by pH and by temperature changes. In order to obtain a sufficient concentration of enzyme to detect significant changes, plasma from patients with uraemia was studied.

(a) pH of inactivation

Technique: The plasma was obtained from heparinized blood. 2 ml. of plasma was adjusted with 1 N.NaOH at room temperature to the desired pH, left for 5 minutes unless otherwise stated, then mixed with 10 ml. (0.4 G.) haemoglobin substrate and adjusted to optimum digestion pH with 1N. HCl. The resulting volume was measured in a graduated pipette and halved. One half was incubated for 60 minutes at 37°C., the other, serving as a blank immediately precipitated in 5 ml. of 10% trichloracetic acid (0.6N). Each half therefore contained 1 ml. of plasma and 0.1 G. of haemoglobin. Volumes were taken into account in the calculations. pH measurements were done with a Cambridge potentiometer.

Results:

The following table shows the details of these experiments.
Experiment 1. Plasma from A.Wi with chronic nephritis, blood urea 354 mgm %. plasma pH 7.19

PLASMA pH adjusted to	INCOBATED AT pH	Hb. (P.U.)	%	% INACTIVATION
11.40	1.70	26		79.5
10.12	1.82	54.2		55
9.58	1.86	56.2		53.5
9.4	1.52	40.2		67.3
9.04	1.76	38		68.5
8.32	1.56	46.2		62
7.19 (not alkalinized)	1.20	61		49
7.19 " "	1.88	121		0

Experiment 2. Plasma from E.Pa, malignant hypertension. Blood urea 280 mgm %

7.34	2.0	46	0
10.37(for 30 mins at 37°C.	2.07	7	85
9.9 (for ½ min.)	2.0	14.8	69
9.5 (for 30 mins at 37°C.)	2.0	15.3	30.5

TABLE NO: 17 pH INACTIVATION OF PLASMA PEPSINOGEN

Discussion

It will be noted that these figures suggest only partial denaturation of the enzyme at pH more alkaline than 8, though both experiments agree in that pH greater than 10.3 causes 80% or more inactivation. It is difficult to see why pH of 10.13 and 9.58 should cause less inactivation in experiment 1 than pH 9.4. It will be noted that at pH 1.20 some inactivation occurs.

The results are somewhat vitiated by the presence of other proteolytic enzymes acting at all pH levels and one cannot be certain that some of the discrepancies are not due to tryptic or catheptic activity.

However, these figures illustrate that the enzyme in plasma, thought to be pepsinogen, cannot be completely inactivated at pH up to 11.40, and only partially inactivated at pH below 10.3. This agrees with inactivation studies of Herriot (1938) (see Chapter II) on pure pepsinogen.

(b) Inactivation by heat.

The plasma used in the last two experiments were also used for some heat inactivation studies.

That used in the first experiment (121 P.U.%) was heated to 70°C. for 10 minutes, and then incubated at pH 1.88 for a further 50 minutes, and then contained 105 P.U.%; showing 13% inactivation.

The plasma used in the second experiment (46 P.U.%) was

(a) Boiled for 3 minutes in haemoglobin at pH 2.0; then contained 0 P.U.%

(b) Heated to 55°C. for 60 minutes; then contained 21 P.U.%

(c) Heated to 70°C. for 10 minutes; then contained 30.7 P.U.%

Therefore heating to 55°C for 60 minutes caused 56% inactivation, to 70°C. for 10 minutes 13% and 32% inactivation respectively and boiling destroyed the enzyme.

This shows that the enzyme measured has a high temperature of inactivation, agreeing with studies on crystalline pepsinogen (Chapter II).

(c) Conversion to pepsin and then inactivating.

Pepsin is denatured fairly rapidly and almost completely at pH over 7.0. By prior acidification pepsinogen is irreversibly converted to pepsin and then behaves as such.

To use this technique the plasma of a patient with extrarenal uraemia was used (Blood urea 260 mgm%, plasma "pepsinogen" 67.3 P.U.%) 1 ml. was adjusted to pH 2.0 with 1 N HCl and then to pH 10.0 with 1 N NaOH and immediately reduced to pH 1.7 and incubated for 60 minutes. It then contained 37 P.U.%, showing a loss of 45% activity.

These experiments, though suggestive, were not conclusive in their proof of the nature of the proteolytic enzyme in the plasma active at pH 1 - 3, and one is forced to fall back on the theoretical proof of its identity with pepsinogen (see above).

Whether the behaviour of the enzyme in the presence of blood proteins or of other enzymes or of other blood constituents is in any way modified from its action in the crystalline state is hard to say, though this might explain the peculiarities. It is possible that the enzyme is not entirely pepsinogen, and that some other enzyme with different physical properties is active in the same pH range, (a conclusion reached by Mirsky et al 1952)

The discrepancy in heat inactivation between 55°C. and 70°C. might be interpreted as the presence of antipepsin, heat-labile at between 55°C. and 70°C., though a more direct experiment designed to show the presence of an antipepsin in the plasma failed to do so (see later).

A more careful study into this aspect of blood proteolytic enzymes is certainly necessary and is being planned. For practical purposes, however, I intend to regard the enzyme as pepsinogen and it will be called such in the following pages.

(iii) The source of plasma pepsinogen.

Except for the observation of Van Calcar (1910) on the presence of "pepsin" in the leucocytes and the disappearance following total gastrectomy, no mention is made in the literature of the presence of pepsinogen in the leucocytes. Barnes (1940) in a careful paper and review makes no mention of it. It appears that the major source of plasma pepsinogen is the fundus of the stomach.

RESULTS.

Experimental evidence to be presented shows that pepsinogen is

formed both in the peptic cells of the stomach, and endogenously in the leucocytes. Evidence will also be presented to show that the gastric pepsinogen diffuses continuously into the blood.

(a) Origin from the stomach

1. In one male patient with a gastric carcinoma who was undergoing a total gastrectomy, blood was taken simultaneously from a peripheral vein and from the left gastric vein as soon as the stomach was exposed. Blood was taken after the end of the operation. The results were:

Before gastrectomy	Peripheral vein blood contained	20.3 P.U.%
	Gastric vein blood contained	46.0 P.U.%
After gastrectomy	Peripheral vein blood contained	0 P.U.%

2. The effect of operations on the stomach on blood pepsinogen

Total gastrectomy in seven patients studied produced in five a complete disappearance of plasma pepsinogen within 48 hours in four and probably in another (D.Ca) In the other patients, one specimen on the 3rd postoperative day contained a small amount 10.5 P.U.% in one and 5 P.U.% on the seventh day in the other. One other patient who had had a total gastrectomy four years before had 25.5 P.U.% plasma pepsinogen.

	-1	2 hrs.	1	2	3	4	5	6	7 days.
D.Bur	20.3	0	-	-	-	-	-	-	-
D.Co	16	12	6.1	-	-	-	-	-	0
A.No	-		-	-	-	10.5	-	-	-
C.Bai	-		-	-	0	-	-	-	-
H.Wa	-		-	-	0	-	-	-	-
E.Du	19		6	0	0	0	-	-	-
C.Wa	-		42.7	-	-	-	-	-	5

TABLE NO: 18 PLASMA PEPSINOGEN AFTER TOTAL GASTRECTOMY.

The immediate effects of partial gastrectomy was studied in four patients. In three, blood was taken 1 hour postoperatively and for at least 3 days after that. Two of the patients showed a rise from the second day to the fourth and fifth days respectively. The other two showed no appreciable change in blood pepsinogen as a result of the operation. These results are set out in Table below. One patient who had had a partial gastrectomy and was bleeding from a jejunal ulcer had a normal level of plasma pepsinogen (22 P.U.%)

	-1	1 hr.	1	2	3	4	5	6	7	8	9days
W.Sa	136	80	152	180	180	240	52	64	68	100	124
J.Ba	59.2	53.2	40.8	52	52	-					
C.O'B	85	67	78	63	63						
W.Tu	31	-	26	62.7	61	31	47	-	-	-	12.5

TABLE NO: 19 EFFECT OF PARTIAL GASTRECTOMY ON PLASMA PEPSINOGEN (P.U.%)

From these results of total gastrectomy it may be concluded that the pepsinogen of the plasma arises from the stomach, and that it probably does so by a continuous process, probably of diffusion directly into the blood, since it disappears within a very short while from the plasma.

From the results of partial gastrectomy it may be concluded that the pepsinogen arises from the portion of the stomach not removed, i.e. the fundus where we know the peptic cells to be mainly situated, although when readjustment has taken place, the plasma pepsinogen does fall somewhat.

The finding of plasma pepsinogen 4 years after a total gastrectomy was rather surprising and suggests that the stomach is probably not the sole source of pepsinogen. This is shown to be so later.

(iv) Distribution of pepsinogen in the blood.

(iv) Distribution of pepsinogen in the blood.

The existence of pepsinogen in the leucocytes was mentioned by Von Calcar (1910) but has not been mentioned since then. When pepsinogen was found in the plasma and it was known to appear in the urine, its distribution throughout the body was investigated. The first line of this investigation was to shw its distribution in the blood.

The following is an example of the distribution in the blood from a 57 year old man (A.Br.) with a gastric ulcer.

	P.U.%
Plasma	54.7
Serum	54.7
Whole blood	88.7
Packed red cells	73.3
White cell layer	140

The white cell layer (buffy coat) was taken from 10 cc. of blood, washed in normal saline twice, sedimented and resuspended in 3 ml. saline. The total white cell count was 600,000 per cu.mm., and the haemoglobin content of this was 9.1 G.%

The following table gives an idea of the relative concentrations of pepsinogen in the various blood fractions per 100 ml. The figures for white cells are for 100 ml. of buffy coat taken from 10 ml. of blood, with an expected mean of about 600,000 leucocytes per cu.mm.

	PLASMA	SERUM	WHITE CELLS	RED CELLS	WHOLE BLOOD
(a)					
A.Br.	54.7	54.7	142	73.3	88.7
A.Wi *	164	152	140	113	-
B.H.	40	-	-	-	49.7
M.D.	28	-	68.3	-	-
MxD	12	-	183	-	-
H.Ho	216	212	-	-	-
(b)					
C.Wa	42.7	-	-	-	153
E.Du	0	-	21	-	-
C.Ba	0	-	130	-	-
D.Co	0	-	55	-	-
H.Wa	0	-	103	-	-

* A.Wi Uraemia.

TABLE NO: 20 DISTRIBUTION OF PEPSINOGEN IN THE BLOOD, P.U. per 100 ml.
(a) Controls
(b) After total gastrectomy.

From the table above one can deduce several things

(1) The pepsinogen concentration of plasma and serum is practically identical. This is to be expected if pepsinogen is in solution a s free pepsinogen.

(2) The whole blood concentration is higher than that of plasma. This is also to be expected since the concentration in the leucocytes is higher than that of plasma.

(3) The concentration in the red cells is similar to that of the plasma.

(4) The concentration in the leucocytes is relatively much higher than

that of any other fraction of the blood. These figures are only approximations and are only semiquantitative.

Several exceptions appear to be applicable to these general conclusions.

(a) In the case of uraemia, exemplified by patient A.Wi above, the plasma and serum concentrations are higher than those of red or white cells in contrast to normals. This is explained later by the hypothesis that only the plasma pepsinogen is filtered through the glomeruli, that of the leucocytes endogensouly formed and not excreted.

(b) In the case of total gastrectomy (the last 5 cases in table20) there is no plasma pepsinogen in four, and in those an appreciable quantity of pepsinogen in the leucocytes. In the fifth there is a relatively greater than expected amount in the red cells compared to the plasma

THE SOURCE OF THE PEPSINOGEN IN THE CELLS

As was shown above, the plasma pepsinogen is labile, varying with gastric activity and arising from the stomach. There is a very rapid decline following total gastrectomy, suggesting that this process of diffusion is a continuous process, as is the clearance of the pepsinogen through the kidney. The presence in the one patient quoted above who had 25.5 P.U.% pepsinogen in the plasma four years after total gastrectomy (this was verified at 2 subsequent operations) indicates that another source of plasma pepsinogen is probably in existence. This patient had an appreciable quantity of pepsinogen

in the leucocytes (between 200 and 300 P.U.%).

The presence of considerable quantities of pepsinogen in the leucocytes in the four cases quoted above, with the simultaneous absence of plasma pepsinogen 2, 2, 7 and 2 days respectively after total gastrectomy, suggests that the leucocytes either retain pepsinogen for some time or alternatively, make it endogenously. It seems more likely, however, that at the end of a week the presence of pepsinogen in the white cells and none in the plasma (the highest plasma pepsinogen in this patient was 12 P.U.% compared to 55 P.U.% found in the white cells a week later) would argue in favour of endogenous formation.

The further evidence in support of this is the reversal of relative values in the case of uraemia, where only plasma levels were increased, and the presence of pepsinogen in the plasma and much more in the leucocytes 4 years after total gastrectomy suggests an endogenous rather than an exogenous source of pepsinogen in the white cells. Is this then the "parasekretion" postulated by Strauss(1928) 25 years ago?; and one wonders whether it is this source which continues in some instances to cause some excretion of pepsinogen after total gastrectomy (see Fig.18) and in pernicious anaemia (see p.243).

(v) Plasma pepsinogen levels.

(a) Normal levels.

20 controls were studied; these comprised 15 males and 5 females composed of hospital staff and ambulant patients free of any gastric symptoms or disease, and with no evidence of renal disease. Excretion levels ranged from 0 to 43.7 P.U.%, with a mean value for the 15 males of 18.46 P.U.%, S.D.±10.8 and for the 5 females 26.9 P.U.%, S.D.±12.7 1 male and 1 female had levels over 40 P.U.%

(b) Plasma pepsinogen in disease.

(1) Gastric ulcers.

11 males and 2 females with gastric ulcers were studied and plasma levels varied from 0 to 55 P.U.%, mean 23.6 P.U.%, S.D.± 10.6 ; in the 2 females the levels were 9.7 and 30.7 P.U.% respectively, mean 20.2 P.U. %. Of these only 2 had more than 40 P.U.% plasma pepsinogen.

(2) Duodenal ulcers.

27 male patients with duodenal ulcers were studied. Excretion levels ranged from 15 P.U.% to 140 P.U.%, mean 60.4 P.U.%., S.D.± 31.2 . Of these 17 had more than 50 P.U.% No females were studied.

(3) Pernicious anaemia.

3 patients studied had levels of 15 P.U.%, 16 P.U.% and 18.3 P.U.% respectively.

It is therefore clear that patients with gastric ulcers are similar to controls in their plasma pepsinogen levels, whereas male patients with duodenal ulcers, irrespective of other factors, have

significantly higher levels of plasma pepsinogen, 63% being above the upper limit of normal (45 P.U.%)

These results agree with the results of Mirsky et al (1952) published since this work was completed.

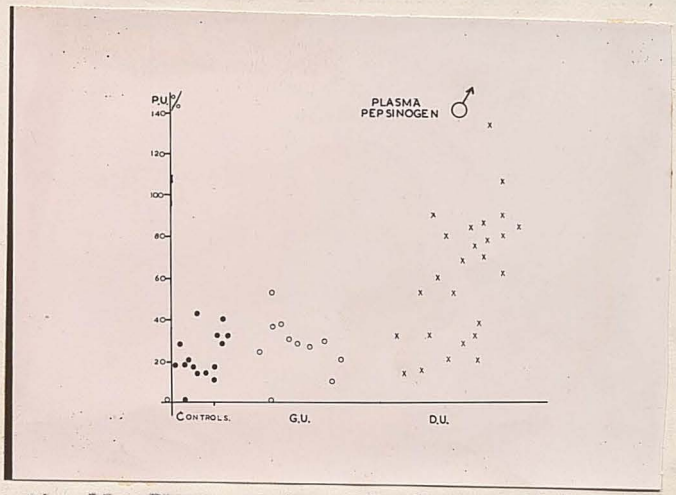


Fig. 15. Plasma pepsinogen levels in controls and gastric ulcers (G.U.) and duodenal ulcers (D.U.).

Significance of difference by "t" test of plasma pepsinogen levels:

Control vs. D.U.	t = 5.2	n = 45	$.0001 < p < .001$
Control vs. G.U.	t = .90	n = 51	$.5 < p$
G.U. vs. D.U.	t = 3.67	n = 38	$.0001 < p < .001$

TABLE NO: 21 COMPARATIVE LEVELS OF PEPSINOGEN IN THE PLASMA IN MALTA.

Hb (P.U.) %	CONTROLS M.	CONTROLS F.	GASTRIC ULCERS. M.	GASTRIC ULCERS. F.	DUODENAL ULCERS.	URAEMIA	PERNICIOUS ANAEMIA
0 - 9	1		1	1			
10 - 19	7	1	1	-	2		3
20 - 29	5	3	5	-	4		
30 - 39	3	-	2	1	4		
40 - 49	1	1	1			1	
50 - 59			1		3		
60 - 79					6	3	
80 - 99					6	1	
100 - 150					2	1	
TOTAL NO.	15	5	11	2	27	6	3
MEAN P.U.%	18.46	26.9	27.6	20.2	60.4	84.5	16.4

No further analyses for age or other factors have been done because of the small number of cases.

The finding of several patients with levels of 0 or nearly 0, especially after total gastrectomy, suggests that Mirsky et al (1952) are not correct in supposing another enzyme. This "other enzyme" is probably an artifact of their long incubation time (see p. 63).

For the rest, these results agree fairly closely with those of Mirsky et al (1952).

(vi) Daily variations of plasma pepsinogen in the individual

Observations extending over several days were made on six subjects and observations on 2 blood samples from 1 day to 1 month apart made on seven subjects. These are summarized in the following table and graph.

TABLE NO: 22 DAILY VARIATIONS IN PLASMA PEPSINOGEN

Patient (Hb.% / P.U.)	1	2	3	4	5	6	7	8days.
1.	59.2	53.2	40.8	52	52			
2.	85	67	78	63	63			
3.	31.3	22.7	25.5	22	17.5	28	26	31.3
4.	24.3	24.3	18.3	17.5	18.3	14		
5.	64.8	57	70	71	105	78.8	73.5	
6.	75	72	65.7	39.8	61	57		
7.	17.5	18.3						
8.	15.7	27						
9.	0	0						
10.	30.7	29.7						
11.	66	26.2						
12	32	32						
13.	18.3	19.2						

As can be seen from these figures there is less daily variation in the individual of fasting plasma pepsinogen than in the urinary pepsinogen excretion. This is understandable, since the latter is dependent on variations in renal function which are in no way related to gastric activity, while the plasma pepsinogen is solely related to the level of gastric activity except in renal disease. It would therefore seem reasonable to estimate only one sample of plasma pepsinogen in the individual, whereas it appears necessary to estimate at least two or three 24 hour samples of urine to arrive at the mean level for the individual.

(vii) Excretion of blood pepsinogen.

As has been shown elsewhere, (pp.123&125) the pepsinogen of the plasma is the labile fraction which changes rapidly with activity of the stomach, or its removal and therefore depends for its maintenance on contunuous diffusion from the stomach. It also depends for its excretion on continued activity of the kidney and is fairly rapidly eliminated after the source of supply has been removed in total gastrectomy. It rises paripassu with other substances, and almost parallel with urea in uraemia.

The pepsinogen of the white cells, however, appears to be formed endogenously and is less liable to change in response to disturbances of gastric activity or renal function. As shown above (p.124) while the plasma pepsinogen is cleared with 48 hours after total gastrectomy, that of the cells is maintained for at least a week, and in one case 4 years after gastrectomy is still present.

In the case of uraemia (A.W.i) the pepsinogen in the plasma exceeds that in the cells, and one assumes that the failure of excretion leads to accumulation in the plasma but not in the cells, possibly because the cells are rather more impervious to the passage of molecules of this size.

In uraemia the plasma pepsinogen rises and as shown later in this chapter in six patients with a mean blood urea of 303 mgm.%, the mean pepsinogen level was 84.5 P.U.%, compared to a normal control level of 25 P.U.% for males and females.

(VIII) The relation of blood pepsinogen to gastric pepsin and urinary pepsinogen.

This subject has been discussed elsewhere (pp.93ff.) where it has been shown that plasma pepsinogen can be correlated positively with gastric pepsin (see graph[14]) but that when the peptic cells are forced to overwork, pepsinogen is withdrawn from the diffusing surface and the blood level drops. This indicates that there is a constant diffusion from the peptic cells to the blood (p.123). It has also been shown that pepsinogen of the plasma arises from the stomach; that of the leucocytes is probably endogenously formed.

Plasma pepsinogen can only be correlated with urine pepsinogen when renal clearance of pepsinogen is taken into account.

IV. THE DISTRIBUTION OF PEPSINOGEN IN BODY FLUIDS.

The distribution of pepsinogen in the body fluids other than gastric juice, blood and urine was investigated, and this information is summarized in the following table.

BODY FLUID	P.U.%
Sweat	17
Cerebro-spinal fluid	29
Pleural fluid (heart failure)	5.25
Oedema fluid (heart failure)	(0 (0
Pus (a) Tuberculous (Lymphocytes 80%) Eosinophils 15%)	0
(b) Supernatant fluid of (a)	0
(c) Pyocyaneas (polymorphs 95%)	133

TABLE NO: 23 PEPSINOGEN IN BODY FLUIDS.

These results indicate that pepsinogen passes through the choroid plexus into the C.S.F. in the same concentration as the plasma, therein behaving rather differently from blood proteins. If one draws again the analogy between choroid plexus and glomerular membrane, it suggests that the concentration in flomerular filtrate would equal that of plasma.

The finding of pepsinogen in sweat confirms Benderskey's(1890) findings - he called this "hidro-pepsin". (He also coined the term "Uropepsin" which is still in use today).

Its presence in very small concentration in pleural fluid and its absence from oedema fluid is in keeping with the transudate nature of these fluids in heart failure.

The absence from tuberculous pus, both cellular and fluid portion, is explained by the presence of a naturally occurring antipepsin (see below) in this pus, or by the natural absence of pepsinogen from lymphocytes.

The presence in high concentration in Ps. pyocyaneas pus is in keeping with its high polymorph content.

This distribution of pepsinogen throughout the body indicates that few membranes are impermeable to this protein. It would appear that there can be no physiological significance to the widespread distribution of pepsinogen in the body, since no part of the body other than the gastric lumen is ever acid enough to supply an optimum pH for the action of pepsinogen. One must assume, then, that pepsinogen is an accidental waste-product when it occurs anywhere but in the stomach.

NATURALLY OCCURRING ANTIPEPSINS.

A crystalline pepsinogen inhibitor has been isolated by Herriot (1941) who has analyzed its various physico-chemical and enzymatic properties. It appears to arise as an intrinsic part of the pepsinogen molecule and to contain about 8 of the 15% of the nitrogen split off in its transformation into pepsin. It is most active at pH 5.7 where it inhibits both the rennet and the proteolytic activity of pepsin. It is rapidly destroyed by pepsin between pH 2.0 and 5.0, maximum near pH 4.0.

This inhibitor therefore is not likely to be operative at the pH at which pepsin is normally assayed and since it does not destroy pepsinogen, one may distinguish this inhibitor from the one to be described later.

Natural antipepsins have ben described before - astrong inhibitor of pepsin was shown to occur in Ascaris by Weinland (1903) and Peczenik and Kawahara (1927) reported an inhibitor in the serum which was thermolabile at 55°C., undialyzable and extractable in the ether phase. They reported that it was constant for a species, varying with diet and parallel to the urinary pepsinogen, being highest in the cat and lowest in the pigeon.

Hamburger (1912) and Oppenheimer (1939) deny the existence of an inhibitor in the serum, and Mirsky et al (1948) failed to detect an inhibitor in the urine.

Experimental evidence.

An attempt to demonstrate antipepsins in the plasma failed to do so. In order to demonstrate this, the plasma of a patient who had had a total gastrectomy 48 hours before and who had no pepsinogen in the plasma was taken. Plasma assay in a patient with gastric ulcer contained 38.5 P.U.% 2 ml. of each plasma were mixed and incubated for 15 minutes and 60 minutes at 37°C. The mixture was then incubated with haemoglobin for 60 minutes as for the other plasma specimens.

1. A.Ro (Gastric ulcer	38.5	P.U.%
2. H.Wa (Total gastrectomy)	0	"
Mixture 1 and 2. 15 min. at 37°C.	19.25	"
60 min. at 37°C.	31.3	"
White cells from H.Wa (500,000 Wbc/cu.mm)	132	"

This indicates that there is no inhibitor in plasma of a patient who has had his stomach removed, but is otherwise normal, and has a normal amount of pepsinogen in the leucocytes.

Antipepsins in pus

Experiments.

(1) One specimen of pus from a tuberculous empyema contained 15% eosinophils and 80% lymphocytes; cells constituted 60% of the total volume of the pus. Assay of this pus failed to reveal any

pepsinogen. The urine of a patient containing 1.30 P.U./ml. was mixed with 1/10 of its volume of pus. The resulting mixture contained no pepsin on assay - and therefore suffered inhibition of 1.3 P.U. in 1/10 ml. pus - 13 P.U. per ml. inhibitor in the pus.

(2) In another experiment with the same pus, but with a urine containing 12.4 P.U. per ml., 6 ml. of the urine was mixed with 4 ml. of the pus and acidified with 2.5 ml. 0.3 N.HCl to reduce the pH to 2.0. 1 ml. of this mixture containing 1 P.U. of pepsinogen and 0.25 ml. of pus was assayed and revelaed no peptic activity and the pus therefore contains the inhibitor equivalent of at least 4 P.U. per ml. If one consideres that all the 6 ml. was inhibited by the 4 ml. of pus, then each ml. of pus contains $\frac{74.4}{4}$ = 18.6 P.U. equivalent in inhibitor per ml.

(3) The supernatant fluid from this pus was assayed for peptic activity, result 0 P.U. Its inhibiting effect was tested on gastric juice pepsin assayed at 584 P.U. per ml. 5 ml. of 1/10 diluted gastric juice (the dilution used for the assay of pepsin) was mixed with 3 ml. of the supernatant pus fluid. The mixture was assayed at 32.7 P.U. per ml. representing the activity of 5/8 ml. of gastric juice (1/10 diluted). Corrected for dilution of gastric juice $(\frac{x10}{1} \times \frac{8}{5})$ the peptic activity of the gastric juice was 525 P.U. per ml. and it had therefore suffered inhibition of 59 P.U. per ml. or 295 P.U. per 5 ml. caused by 3 ml. of pus fluid, each ml. of pus therefore causing 98.3 P.U. equivalent of inhibition.

(4) A specimen of pyocyaneas pus failed to cause any inhibition of urinary peptic activity.

Conclusions.

One is left to speculate on the nature of this inhibitor which is active at pH of assay (2.0) which is fairly strong (at least 98.3 P.U. equivalent per ml.) and which occurs in tuberculous pus - this one containing 80% lymphocytes and 15% eosinophils - both in cellular and in the fluid portion of the pus. It is absent in polymorph pus and from the plasma.

From the pH range of its action and the fact that it is not destroyed at acid pH by the pepsin, one concludes that it is not identical with the pepsin inhibitor described by Herriot (1941) (see above).

SECTION VI. THE RENAL BARRIER (in relation to protein excretion in general and pepsinogen in particular.

There has been more speculation than fact about the exact mode of excretion of pepsinogen by the kidney. Neumeister (1888) speculated about the role of the kidney in excretion and concluded that because he could demonstrate no "pepsin" in the blood but could in the urine, that the enzyme circulated in an inactive form in the blood combined with an inhibitor, and that this complex was split and the enzyme activated in the tubules.

The first reference to any disturbance of urinary pepsinogen was a statement by Van Noorden (1893) that its excretion was increased in nephritis.

Most authors, however, agreed that nephritis caused a diminution of urinary pepsinogen (Strauss 1926, Rothschild 1930) and that it caused a rise in plasma pepsinogen (Gottlieb 1924); Loeper and Debray (1922) found an increase in the blood of a dog whose ureters were tied.

More recently, Bucher (1947) has stated that pepsinogen is eliminated as other waste products and that "the molecular weight of pepsinogen (42,000) presents no problem to the kidney, since it is smaller than egg albumen (44,500) and haemoglobin (69,000) both of which may be eliminated by the normal kidney". Mirsky (1950b) in answer to a question from Homer Smith as to whether pepsinogen might not be re-absorbed in the renal tubules to a slight extent

as are a large number of other proteins said "I might state that we have tested the excretion of pepsinogen in dogs and it does not appear that reabsorption of pepsinogen occurs". The only other recent authors on pepsinogen excretion, viz. Janowitz, Levy and Hollander (1950) made no comment other than estimating the blood urea in all cases to exclude renal disease.

It was evident that this problem was far from settled and needed close investigation. The results of this investigation have shown that the kidney does in fact play an active role in the excretion of pepsinogen, and that pepsinogen excretion by the kidney is merely one facet of the whole problem of protein excretion by the kidney, a subject on which there has been a great deal of speculation and which only now is beginning to become understood. As will be shown later, this protein, pepsinogen, is filtered through the glomerular membrane and partly reabsorbed in the tubules, in a manner analagous to other threshold substances like glucose, and is merely representative of the "threshold proteins" dealt with in like fashion.

It would perhaps be best to review the evidence pertaining to the modern views on protein filtration and reabsorption before proceeding to the experimental evidence specifically relating to pepsinogen.

Protein filtration and reabsorption by the kidney.

A search of the literature reveals that the idea of protein filtration and reabsorption is by no means new and preceded, but

was unfortunately temporarily superseded by the theories of Cushny (1917,(1926).

The classical thesis of Cushny held that the normal glomerular membrane is impermeable to protein and that protein only passed through damaged capillary membranes, after which it remained uninfluenced by either secretory or resorptive activities of the tubule.

The more modern views based on experimental evidence and observation in disease hold that protein is filtered through the glomeruli both in disease and in health and that this filtered protein is then resorbed in the tubules, probably the proximal convoluted tubule, and that variations in proteinuria depend on the tubular activity. This tubular activity is not a simple stuffing of the tubule cells with protein but rather an active, dynamic transfer of material from the tubular urine to the blood aided by enzymatic processes (Rather 1952)

Blood proteins.

Anatomically the glomerulus resembles the choroid plexus in having a blood/fluid barrier of both endothelial and epithelial layers. The C.S.F. protein is in the neighbourhood of 20 mgm. % and Dock (1942) found that the urine from kidneys perfused with ice-cold serum to paralyze tubules contained 15 - 22 mgm.% of protein. If no protein were reabsorbed large quantities would appear in the urine. That this does not do so normally shows that it is reabsorbed in the tubules.

Other proteins.

Claude Bernard (1859) in observing proteinuria and excretion of injected egg albumin and of haemoglobin was the first to suggest that the mechanism was similar to glycosuria - a remarkably prescient view held at the time pepsinogen was only thought of as a theoretifal possibility. Ponfick (1875) showed that the red colour of the urine in transfusion reactions was due to haemoglobin and not to blood and determined the levels of haemoglobin in the serum necessary to produce this.

Cushny in his book published in 1917 and again in 1926 set the tone of modern renal physiology. He insisted that proteinuria proved a renal lesion visible or invisible in the glomerulus, though he was aware of the observations on haemoglobinuria and alimentary proteinuria in normal subjects. He passed these off as indicating that both eggewhite and haemoglobin caused glomerular damage because they were eliminated as foreign protein. This explanation completely ignores the possibility of normal resorption of protein by the tubules. De Haan (1922) showed that trypan blue which is bound to protein does not appear through collodion membranes but that it does appear in the urine, and took this to mean that protein was in fact filtered through the glomerular membrane, and that the glomerulus did not therefore behave as an ultra-filter. He further believed that because the dye appeared in the urine hot bound to protein, the protein was removed in the renal epithelium by enzymatic digestionl

Bayliss, Tookey-Kerridge and Russell (1933) showed by extensive investigation that there was no chemical or histological data to support Cushny's theory that the kidney selectively holds back body protein and excretes foreign protein through the glomerular membrane. They found that gelatin (m.wt. 35,000), Bence-Jones protein (m.wt. 35,000), egg-white protein (m.wt. 44,000) and haemoglobin (m.wt. 69,000) were all freely excreted while blood proteins (albumin and globulin), edestin and casein were held back. They explained this phenomenon on a basis of molecular weight, suggesting that 60,000 was the limit of glomerular excretion of proteins. They found no explanation in this theory of how haemoglobin was excreted and the possibility of tubular reabsorption held no place in their explanation. They were unable to demonstrate any histological damage in the glomeruli to support Cushny's theories. A probable explanation of their failure to obtain any excretion of large molecules are the low plasma levels they produced. Brull (1931) by ingenious cross-circulation experiments, was unable to demonstrate any evidence to support the glomerular damage theory of Cushny by the "foreign" proteins.

EVIDENCE OF TUBULAR RESORPTION OF PROTEINS.

(1) Clearance Studies.

Pearce, Austin and Eisenbrey (1912) observed the "threshold" of haemoglobin to be about 150 mgm. per 100 ml. of plasma, later confirmed by Lichty, Havill and Whipple (1932) and Newman and Whipple (1932), and later still by Yuile, Steinman, Hahn and Clark (1941). The latter three papers (from Whipple's laboratories)

showed that when haemoglobin appeared in the urine there was a proportional deposition of iron-containing pigment in the proximal tubules, whereas at "sub-threshold" levels when no haemoglobin appeared in the urine, no pigment appeared in the proximal tubules. It hardly appears tenable to assume that an actual threshold exists below which haemoglobin is held back by the glomerulus, and one believes that sub-threshold levels merely indicate the limit which the tubules are able to manage without breakdown products appearing in the tubular epithelium. That this is due to an actual breakdown of haemoglobin in the tubular epithelium is proven by the identity of the iron-containing pigment in the epithelium as haemosiderin (Rather 1952). Repeated daily injections of haemoglobin cause a lowering of the "threshold" indicating a probable over-loading of the tubular epithelial mechanisms.

Monk and Yuile (1940) were the first to estimate clearances on filtration and resorption of a protein. They estimated simultaneous haemoglobin and endogenous creatinine clearances and found that with plasma levels between 250 and 1,000 mgm.%, haemoglobin excretion was 3% that of creatinine, i.e. that about 97% was reabsorbed in the tubules. This was later confirmed by Dock (1942) using the ice-cold serum perfusion technique.

Lippman, Green and Oliver (1951) showed that the reabsorption of protein in rat kidney can be calculated to conform to a mathematical formula. With the limited results at my disposal to date on pepsinogen I am unable to say whether pepsinogen follows their formula, since that is based on maximum rates of reabsorption. They found that under

those conditions haemoglobin excretion (E) will be proportional to the product of exogenous creatinine clearance (F) and serum levels of haemoglobin (S) in a straight line with the slope G, assuming the maximum tubular reabsorption for haemoglobin (Tm Hb) to be constant.

Therefore $E = - Tm\ Hb + G\ F\ S$.

With this formula I found G to vary in 1 patient (J.M.) under constant daily observation from 2.17 to 4.9 with an average figure of 3.34. The small range of values for G indicates that the formula might be applicable to pepsinogen.

There is little doubt in view of these findings that both in experimental animals as proved by haemoglobin excretion and in normal humans as shown by the results in this paper, that protein is filtered through the glomerular membrane and reabsorbed in the tubules.

2. MORPHOLOGICAL EVIDENCE FOR TUBULAR REABSORPTION OF PROTEINS.

Well over 100 years ago, pathologists began noticing discrete spherical intracytoplasmic bodies in the renal tubular epithelium, and much was written in the German literature on the nature of these bodies. It was first thought to be either a degenerative change, later to be evidence of tubular secretion, and later still, evidence for tubular reabsorption of proteins. The point was not settled until Gerard, Cordier and Lambert (1933, 1933 and 1934) showed in frogs and salamander kidneys that these droplets indicated resorption rather than degenerative or secretory processes in the tubular

epithelium. The subject is well reviewed and commented on by Rather (1952). The process of resorption is evidenced by the droplets was looked upon as a static one but the work of Dock (1942) and of Oliver (1950) shows that the process is a dynamic and continuous one. Dock (1942) pointed out that if the filtrate contained only 15 mg. of protein per ml., 27 G. a day would appear in the urine. Since only traces are found, the rest must have been absorbed, and therefore a continuous dynamic process. Oliver showed that the droplet formation is closely connected with the mitochondria in the tubules. From the work of Addis et al (1950) it seems as though the adrenal hormones play some part in maintaining induced proteinuria.

Briefly restated, it appears that protein normally does pass into the glomerular filtrate, partly depending on particle size, possibly also on particle shape, but not on the nature of the protein. It appears unlikely that there is any glomerular threshold but rather that it is a threshold for the whole kidney depending mainly on the resorptive capacity of the tubules for the particular protein, whatever that in turn depends on, and that this resorption occurs in the proximal convoluted tubule and is closely related to the mitochondria.

The exact mechanisms at work can no more than be hinted at by the available evidence. Much of the evidence perforce has to be collected under abnormal conditions and it is not often that physiological and morphological observations can be correlated in the same kidney, and many of the morphological changes are probably ultra-microscopic.

RENAL EXCRETION OF PEPSINOGEN

Experimental evidence.

The evidence to be presented fits in well with the theories and conclusions relating to other proteins.

The most direct evidence to be presented is the determination of the amount of pepsinogen reabsorbed by simultaneous determinations of endogenous pepsinogen and endogenous creatinine clearances in normal men calculated from the formula $\frac{UV}{P}$, Where U= urinary concentration per 10^0 ml., V= urine volume in mls. per minute and P plasma concentration per 100 ml. This formula of Van Slyke has been accepted as applying to both substances, since neither is excreted by the tubules. The creatinine clearance (Csc) has been taken as the glomerular filtration rate and the percentage reabsorption calculated from the formula $100 - \frac{CSP}{CSC} \times 100 =$ % reabsorbed. This puts the figure of reabsorption at between 85 and 95% of the amount filtered through the tubules. Precedence for using this method can be taken from Monk and Yuile (1940) who used the same technique for haemoglobin clearance and reabsorption studies.

This estimation was performed on two patients. The first, H.B. was a 49 year old man recovering from a myocardial infarction in the 5th week and with no signs of renal disease or of cardiac failure. Three consecutive 24 hour urines and two consecutive fasting blood specimens were analyzed for pepsinogen and creatinine. The urines gave the following results: 242, 220 and 186 P.U. % and 113 and 116 mgm % creatinine on two of the urines. The plasma

pepsinogen levels were 49.3 and 45.3 P.U.% respectively and the plasma creatinine 0.84 mgm % on one. These figures are given to show the relative constancy of both substances. The calculated creatinine clearance = 128 ml./min. (= Glomerular filtration rate), the pepsinogen clearance = 8.25 ml./min.

Therefore percentage pepsinogen reabsorbed $= 100 - \frac{CSP}{CSC} \times 100$

$= 100 - \frac{7.25}{128} \times 100$

$= 100 - 5.7$

$= 94.3\%$ reabsorbed.

In a second subject, a normal control several consecutive days were observed and gave the following results:

Day of observation.	Creatinine clearance (GFR)	Pepsinogen clearance	% Pepsinogen reabsorbed.
1	57	19.4	66
2	73.5	9.6	81.2
3	88	15.2	82.8
4	99	22.7	77
5	66.5	4.2	93.7
6	68	11.9	82.75

Table 24. : Endogenous pepsinogen & creatinine clearances and calculation of tubular reabsorption of pepsinogen in a normal subject in 6 successive days.

These figures show a relative constancy from day to day and amply confirm the modern views on protein filtration and reabsorption in the kidney.

Since some criticism might be levelled at the use of endogenous creatinine in the estimation of the G.F.R. these experiments should be repeated using inulin clearances instead. However inaccurate the creatinine clearances, the pepsinogen clearances, as will be shown, are relatively small, varying normally from 5 to 25 ml./minute and if one assumes the G.F.R. to be about 120 ml. per minute, one can predict the percentage reabsorption of pepsinogen to be between 80 and 96% of the amount filtered through the glomeruli.

In attempting to place the site of pepsinogen reabsorption, one has to go no further than the work of Peczenik (1927) who presented an exhaustive study of the relation between urinary pepsinogen and starvation and feeding. In attempting to confirm experimentally in animals the observations of an increase in urinary pepsinogen in human diabetes mellitus, Peczenik induced phloridzin glycosuria in rats. Normal rats respond to 48 hours starvation by practically complete disappearance of pepsinogen from the urine, but such a rat given phloridzin passes double the normal amount of urinary pepsinogen. He wrongly concluded that this confirmed the finding of an increase of urinary pepsinogen in diabetes. (Diabetics, in fact, excrete a normal amount of urinary pepsinogen. He also determined the effect of uranium nitrate poisoning on frogs and found that small amounts caused an increase and large amounts a decrease of urinary pepsinogen excretion.

Since phloridzin poisoning is known to damage the proximal tubules, the analogy between "threshold" glucose and pepsinogen as a "threshold" protein becomes stronger and places the site of tubular

reabsorption in the proximal convoluted tubules.

Pepsinogen clearances were determined in 67 instances and the accompanying graph shows these clearances plotted against plasma pepsinogen levels. This graph shows some linear correlation when plotted on log probit paper, a high clearance occuring with low plasma pepsinogen and vice versa, indicating possibly some mechanism whereby the reabsorption of pepsinogen is controlled according to blood level. This graph (Fig. 16) shows that post-operative clearances are different from normal clearances.

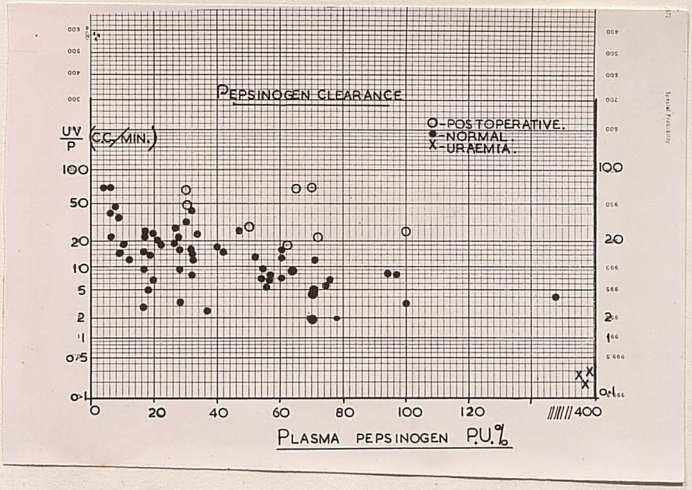


Fig. 16. Relation between pepsinogen clearance by the kidney and plasma pepsinogen levels plotted on log probit paper.

The clearances repeated in patients shows a fair degree of constancy, perhaps more so than the urinary levels of excretion. No relation with disease of the stomach or duodenum has been found. There were 6 patients with gastric ulcer, 4 with duodenal ulcer and 6 controls.

TABLE NO: 25 RENAL CLEARANCE OF PEPSINOGEN IN Ml./min. (See text).

GASTRIC ULCER	DUODENAL ULCER	CONTROL
7.5	16.7	3.2
12.4	6.9	9.3
16.3	(6.6	(18.3
12.9	(4.25	(22
16.4	(20.6	(7.4
(28.6	(22.5	(7.7
(11.8		(19.4
(16.8		(9.6
		(15.2
		(19.2
		(21.6

Bracketed figures are consecutive readings on the same patient. In addition, a number of serial observations were made on two patients who received A.C.T.H. or cortisone; one was later operated on (see below).

The mean clearance for these 16 patients was 14.1 ml./minute and

on an assumed G.F.R. of 120 ml./min., reabsorption on the average would be 88.7%

These figures correspond fairly closely with pepsinogen clearances estimated from Gottlieb's (1924) figures of urine flow, plasma and urine pepsinogen using a different method of peptic assay. Four clearances could be estimated from his figures and these are 16.5, 18, 19 and 14.2 ml./minute. No other figures are available in the literature as only Gottlieb (1924) estimated plasma and urine pepsinogen with any degree of reliability.

Clearance studies under various physiological conditions and in renal disease complete the picture of the mechanism of excretion of pepsinogen by the kidney.

Uraemia.

The effect of uraemia on blood pepsinogen has been shown by Loeper and Debray (1921) and by Gottlieb (1924) and the depression of urinary pepsinogen was noted by A.Rosenthal (1911) (Quoted by Rothschild 1930).

In 6 patients with uraemia, the plasma pepsinogen was raised well above normal, which is 23 P.U.% The following table shows the results and confirms retention of pepsinogen in the plasma in uraemia.

Patient.	Blood urea mgm.%	Plasma pepsinogen P.U.%
C.I.	244	61
I.P.	180	63.5
R.P.	260	48
J.H.	600	213
A.W.	354	84
C.O.	180	67.5
Mean	303 mgm %	89.5 P.U.%

TABLE NO: 26 PLASMA PEPSINOGEN IN URAEMIA

Clearance studies were done on 2 patients with terminal uraemia due to chronic nephritis, J.H. and A.W.

The pepsinogen clearance for J.H. was 1.28 ml./minute and for A.W. on two successive days 0.066 and 0.230 ml./minute respectively. Here the mechanism is probably not an alteration in reabsorption but a reduction in the filtering surface of the tubules to almost vanishing point.

In compensated uraemia (chronic uraemia) where the urine output is reasonably well maintained and the excretion of waste-products maintained at equilibrium with the blood urea elevated to two to three times normal, pepsinogen clearance appears to be unaffected. One such patient was studied (I.P.) and the clearance in this case was 17.9 ml./minute. This reading is somewhat complicated by the coincident proteinuria (2 to 3 G./day). Whether this indicates any general action by the tubules in rejecting protein is

difficult to say and may account for the apparently normal clearance. One would have to observe a simultaneous G.F.R. to answer that question.

Nephrosis

One patient was studied with an amyloid nephrosis. His urinary pepsinogen was 357 P.U. $\frac{Hb}{hr}$ and his blood pepsinogen 20.3 P.U. $\frac{Hb.}{\%}$ and the pepsinogen clearance 32.5 ml. per minute. This would seem to indicate a diminished reabsorption of pepsinogen and would conform to the general pattern of a nephrosis. He was excreting 4 to 6 G. of protein in the urine per 24 hours.

Diabetes Insipidus.

One patient studied was passing 6 litres of urine per day. The urinary pepsinogen was normal in this patient. 286 P.U. $\frac{Hb.}{hr.}$

Diabetes Mellitus.

The urinary excretion in 4 patients with this disease was normal and averaged 194 P.U. $\frac{Hb.}{hr.}$

Forced Diuresis

The effect of mersalyl diuresis was studied in 3 patients and the following figures of urinary excretion appear to indicate that forced diuresis can increase the output of pepsinogen.

T.W.	Days.	Urine ml./hr.	P.U./hr.	
	1	130	216	
	2	54	282	
	3	39.5	316	
	4	41.5	380	
	5	600	550	1st 4 hrs. after mersalyl
		150	275	Next 20 hours.
	6	66	292	
	7	120	226	
J.P.	1	25	325	
	2	31.5	232	Mersalyl
	3	85	714	

In a third patient mersalyl had no effect on pepsinogen despite a normal diuretic effect.

Effect of operation

As is pointed out later in this chapter (p. 201) partial gastrectomy has the effect of temporarily increasing the output of pepsinogen in the urine to levels not met with under any other conditions. In other operations, this effect is seldom seen. As is pointed out in that section, when the pepsinogn excretion is increased to those levels, part of the mechanism of that increase is the increased output from the fundal remnant of the stomach, but the main mechanism for increasing the urinary excretion appears to be a

decreased tubular reabsorption of pepsinogen and the pepsinogen clearance rises from the normal of about 14 ml./minute to 60 to 70 ml./minute. Unfortunately G.F.R was not measured post-operatively and no good reference to this study has been detected in the literature. If one assumes that the G.F.R. does decrease that is even more in support of an increased tubular reabsorption.

The Effect of Cortisone and A.C.T.H on renal excretion of pepsinogen.

The effect of the adrenal steroids in pepsinogen excretion in this study has been shown to be variable (see later) contrary to the work of Gray and his colleagues (1951) who claimed an invariable increase after administration of these hormones.

Of 4 patients studied under the influence of these drugs (se p. 201) one showed a slight increase in pepsinogen excretion without an increase in blood pepsinogen, and this was coupled with slight increase in pepsinogen clearance; partial gastrectomy later caused an excretion peak of 2460 P.U./hr. and a clearance of 67.2 ml./minute in this patient.

(See Table No. 28 overleaf).

Days.	Urine pepsinogen Hb. P.U. /hr.	Plasma pepsinogen P.U.%	Pepsinogen clearance UV/B	
1	190	33	6	
2	355	31.5	19	50 mg.A.C.TH
3	260	23	19.2	
4	345	25.5	21.6	
5	187	21.8	14.5	200 mg. Cortisone.
6	251	18	24.1	
7	300	28	17.7	
8	300	-	-	
9	400	26.2	28.6	
10	420	26	28.0	
11	327	23	17.4	

TABLE NO. 28 EFFECT OF A.C.T.H. AND CORTISONE ON PEPSINOGEN CLEARANCE.

Another patient who had 100 mg. Cortisone per day for 5 days showed no increase in excretion or change in clearance. Two other patients who received in 7 days 560 mg. A.C.T.H., and 1400 mg. Cortisone respectively showed no change in renal clearance as a result of these hormones (see p.).

In the odd patient, therefore, who does show an increase of urinary pepsinogen, one might assume this to be an effect on the renal mechanism of excreting this substance and not on the production of pepsinogen. As will be shown later, A.C.T.H. and Cortisone in one patient had no effect on gastric secretion.

SUMMARY

Experimental evidence has been presented which shows that pepsinogen, a protein with a molecular weight of 42,000, is filtered through the glomerular membrane and largely reabsorbed in the tubule, probably the proximal convoluted tubule. This confirms the other evidence from the literature suggesting that this is similar to what happens to most proteins in the kidney. Because of the simplicity and reliability of pepsinogen estimations, further study of this substance will be useful in clarifying the mechanism of protein excretion by the kidney, both in health and in disease. It is further suggested that many of the variations observed under various physiological and pathological conditions should be evaluated with this fact in mind.

SECTION VII. PEPSINOGEN IN THE URINE.

This section deals with pepsinogen as it is found in the urine under the following headings:

1. Introduction.
2. Comparative biology.
3. Identity of enzyme.
4. Origins and sources of pepsinogen in the urine.
   (a) Site of formation.
   (b) Relation to gastric activity.
   (c) Stimulants of gastric secretion.
   (d) Effects of diet and starvation.
5. Relation to renal function and to physical properties of urine.
   (a) Renal function
   (b) Relation to volume and concentration of urine.
   (c) Relation to PH of urine
   (d) Relation to chloride excretion.
6. (a) Patterns and variations.
   (b) Normal levels; effects of age and sex.

INTRODUCTION

In 1861 Brücke described the presence of an enzyme in the urine which was proteolytic in highly acid medium and he believed this to be pepsin absorbed from the gastric lumen. Until some years after the discovery of pepsinogen (Langley 1881) it was believed that the urinary enzyme was pepsin. Schmappauf (1888) however, believed that the enzyme was not pepsin, but pepsinogen, since it could be found in alkaline urines (of the horse) that would inactivate pepsin. Gehrig (1886) two years before postulated that pepsinogen was absorbed directly from the peptic cells of the stomach into the blood and reached the urine in that way. Benderskey (1890) was still uncertain of the identity of the enzyme and called it "Uropepsin", a misnomer which has persisted to date.

The German clinicians of the late 19th and early 20th centuries saw in the estimation of urinary pepsinogen a new diagnostic method and investigated the variations in this enzyme in relation especially to achlorhydria and the diagnosis of cancer. Following Gottlieb's (1924) fine paper describing his attempts to correlate gastric activity with blood and urinary pepsinogen, a few sporadic papers on urinary pepsinogen were published in German till 1930, when Rothschild discredited the diagnostic significance of "Uropepsin". No further work was done until the publications of Farnsworth, Speef and Alt (1946) and of Bucher (1947) reawakened interest in the subject and these were followed by a series of papers relating mainly to the diagnostic significance of urinary pepsinogen excretion in peptic ulcers. Part of this recent work related to the physiology of this

enzyme and brought us to the point of knowing its source, properties and some of the variations in disease.

COMPARATIVE BIOLOGY

It has been shown that while trypsin or trypsinogen is normally absent from the urine, it may appear in the urine of severely ill animals but not in the dog or in man (Ammon and Chytrek 1939). Pepsinogen, however, is always present in the urine of normal man and animals with a few exceptions. The following animals were found to excrete pepsinogen; man (Brücke 1861); dog and rabbit (Gehrig 1886); Horse (Schnappauf 1888); cat (Frouin 1904); and in 1927 Peczenik and Kawahara reinvestigated the excretion of pepsinogen in animals. The following table is taken from their paper:

TABLE NO: 29 URINARY PEPSINOGEN IN VARIOUS ANIMALS. (Peczenik and Kawahara 1927).

Animal	Urine reaction to litmus	Dilution of urine and degree of peptic activity. 1/1	1/2	1/4	1/8	1/16
Cat	Acid	++++	++++	++++	++++	++++
Guinea pig	Strongly alkaline	++++	++++	+++	+++	+++
Man	Weak acid	++++	++++	+++	+++	++
Dog	Weak acid	++++	++++	++++	++++	+++
Rat	Acid	++++	++++	++++	+++	±
Horse	Alkaline	++++	++++	+++	+	-
Mouse	Strongly acid	++++	+++	++	-	-
Hedgehog(Hibernating & starving)	Neutral	+++	++	++	-	-
Pigeon	-	+++	++	++	-	-

TABLE NO. 29 (cont).

ANIMAL	Urine reaction to litmus	Dilution of urine and degree of peptic activity. 1/1	1/2	1/4	1/8	1/16
Rana esculenta)	(Winter conditions)) alkaline	-	-	-	-	-
Rana temporaria)		-	-	-	-	-
Salamander	-	-	-	-	-	-

Key = No activity.

++++ = Maximal digestion.

The finding of high urinary pepsinogen in cats and moderate amounts in dogs was later confirmed by Bucher (1947) and by Mirsky et al (1948) respectively. The absent excretion of pepsinogen in cold-blooded animals (frog and salamander) in winter is reversed in summer, and by feeding these animals. Intraperitoneal injection of pepsin in the apeptic frog in winter will result in increase of urinary peptic activity, indicating that in the frog the enzyme may circulate and be excreted as such.

The level of urinary pepsinogen in the frog parallels its peptic secretion in the peptic cells of the oesophagus where the frog normally has peptic cells (Peczenik and Kawahara 1927).

In addition to their report on peptic activity in the urine, Peczenik and Kawahara (1927) found that the sera of all animals contained an antipepsin parallel to the level of urinary pepsinogen. Later work failed to confirm this finding (see p. 138).

THE IDENTITY OF "UROPEPSIN"

The term "uropepsin" is used pro tem to describe the proteolytic enzyme in the urine active in a highly acid medium which was discovered by Brücke (1861) and named by Benderskey (1890).

It was quite natural that an enzyme behaving like pepsin in the urine should be considered to be pepsin before the discovery of pepsinogen by Langley (1881). Langley had shown that pepsin was inactivated in alkaline medium, but that pepsinogen was not. Gehrig (1886) believed that the pepsinogen was directly absorbed from the peptic cells of the stomach. Schnappauf (1888) concluded that because the urine of the horse was alkaline and contained "pepsin", it must exist in the proenzyme form, i.e. ppsinogen.

Later experimental evidence on the inactivation of the urinary enzyme did little to clarify its identity. Grober (1904 and 1905) and later Ellinger and Scholz (1910) from alkali and heat inactivation studies believed that the urinary enzyme was a mixture of pepsin and pepsinogen. Gottlieb (1924) knowing that pepsinogen was converted to pepsin at pH below 5.0, argued that the enzyme must be pepsinogen in its free state, since pH of urine in man never dropped below 5.1 (an important observation later to be confirmed again and again).

More recent evidence on the identity of the urinary enzyme with pepsinogen is more valid since one has the absolute standards of comparison with the crystalline pepsinogen isolated by Herriot (1938). Bucher (1947) repeated the inactivation studies of Ellinger and Scholz (1910) using the more accurate haemoglobin method of assay.

She mixed two urines (of pH 7.41 and 7.64 respectively) with high pepsinogen content 1:1 with HCl, NH_4OH, Na_2CO_3 and H_2O for 1 hour; these were then incubated at pH 1.94 and 3.60. She found that as the pH of the initial mixture was advanced to the alkaline side of 7.0 inactivation began; thus at pH 7.64 5% activity was lost, at pH 8.5 76% activity was lost, at pH 9.0 90% activity was lost, and at pH 9.4 and over all activity was lost. Comparing this to purified Merck pepsin and gastric juice diluted in urine, she found that at pH 6.29, no loss of activity occurred and at pH 7.66 complete loss of activity of the pepsin occurred when the mixture was subsequently assayed at acid pH 1 hour later. This confirms that pepsin is destroyed at pH 7.66 and not at 6.29 and that the urinary enzyme behaves like crystalline pepsinogen (see p. 22) and not like pepsin.

Comparing the inactivation of the urinary enzyme at only pH 9.4 with that of crystalline pepsinogen at about pH 10.0 and of pure pepsin or gastric juice at pH 7.6, Bucher (1947) considers her case proven and concludes that the urinary enzyme is pepsinogen. However, the evidence is not conclusive.

Comparing the pH of optimum digestion Mirsky et al (1948) conclude that all that digests between pH 1.5 - 3.5 is pepsin either pre-existing as pepsin or as pepsinogen. In order to determine experimentally which it was, they repeated all the experiments that had previously been done on the parenteral administration of pepsin (Voight 1923) and found, as she did (Voight was the first

to use a non-toxic preparation of pepsin prepared by Witte of Rostock) that intravenous administration of pepsin produced no increase in the "uropepsin" of the dog. (Voight (1923) also showed (using the dog and guinea pig) that not only was there no increase in "uropepsin" but that after large doses of pepsin intravenously into the animal no increase in proteolytic activity of the serum could be detected within a very few minutes.

Mirsky et al (1948) then injected purified pepsinogen intravenously into the dog and produced a marked increase in "uropepsin". This was repeated a few days later and showed the same effect.

One may justifiably conclude therefore that "uropepsin" is pepsinogen in the urine and that the term "uropepsin" should be dropped in favour of "urinary pepsinogen". The term "uropepsinogen" has been suggested but the word is ugly and unnecessary.

THE ORIGINS AND SOURCES OF PEPSINOGEN IN THE URINE.

The origin of the urinary enzyme was considered to be the stomach by the man who discovered urinary pepsinogen (Brucke 1861). He believed that it was reabsorbed as pepsin from the gastric lumen or intestine, a view held for about 25 years. Langley (1881) then showed that pepsin was destroyed in the alkaline small intestine and demonstrated pepsinogen in the cells of the stomach glands. Schnappauf (1888) and Gehrig (1886) thought the enzyme to be pepsinogen and the latter believed it to be absorbed directly from the peptic

cells. This view is still valid today.

Definite evidence for its origin from the stomach came from the work of Benderskey (1890) and later Matthes and Grohe (1903) and of Frouin (1904) who performed several similar experiments on dogs. Total gastrectomy caused complete loss of the enzyme from the urine; oesophago-duodenal anastomosis, leaving the stomach in situ and draining exteriorly produced no loss of urinary pepsinogen, whether the pouch was drained continuously or only emptied once in 24 hours (Frouin 1904). Complete removal of the gastric mucosa, leaving the muscularis coats only, caused disappearance of the urinary enzyme.

Subsequently, numerous authors have shown the absence of urinary pepsinogen after total gastrectomy in man (Janowitz, Levy and Hollander (1950) rats (Balfour et al 1948), and cats (Bucher and Ivy (1947). In one of four cats, Bucher and Ivy found persistence of traces of pepsinogen innthe urine up to 88 days after total gastrectomy.

Clinical observation on disease of the stomach supports the experimental evidence that the stomach is the source of the enzyme. There is a diminution or disappearance following widespread gastric carcinoma and pernicious anaemia (Podore et al 1948; Janowitz et al 1950; Farnsworth et al 1946; Rothschild 1930). In pernicious anaemia, Strauss (1926) found some urinary pepsinogen present, a fact he could not relate to the achylia and postulated a "parasekretion" - another source of pepsinogen. This view is dealt with in the next chapter (p.243). In gastric atrophy there is a practically

complete disappearance of pepsinogen (p. 251).

Other experimental evidence relates partly to the source and nature of the enzyme as well as to how it is transferred from the stomach to the urine.

Langley (1881) showed that pepsinogen was destroyed at alkaline pH and subsequent experiments confirmed that the origin of the urinary enzyme was not from absorption from the bowel lumen - this was first shown by feeding of crude pepsin by mouth to man (Schnappauf 1888), to gastrectomized dogs (Pechstein 1911, Wilenko 1908) and feeding of gastric juice to a gastrectomized dog (Ellinger and Scholz 1910).

The intravenous administration of pepsin was not done successfully until 1923 when Voight had the first non-toxic pepsin prepared for injection. Previous workers had always found that pepsin intravenously caused the death of their animals (this was due to protein shock from adulterating proteins) and Benderskey (1890) who found this so, believed that pepsin was poisonous and ended an otherwise good paper by warning against the indiscriminate use of this "poison" in elixirs, pastilles and mixtures. Apparently the patent medicine manufacturers had nothing to learn in 1890, even in Russia.

Voight (1923) found, as did Mirsky et al (1948) that intravenous pepsin produced no increase in urinary pepsinogen. She also found that pepsin was destroyed at the pH of the blood very rapidly.

Only in the frog does parenteral administration of pepsin (into

the lymph sac) cause an increase of urinary enzyme (Peczenik and Kawahara 1927) but whether this enzyme is pepsin, or whether the pepsin as a protein causes an increase of oesophageal secretion of pepsin in the hibernating frog is not clear.

Certainly in mammals parenteral pepsin produces no increase in urinary pepsinogen and the pHof the blood acts as a most effective barrier to the transition of pepsin from the stomach to the kidneys as pepsin.

Finally Mirsky et al (1948) showed that pepsinogen orally had no effect on the urinary enzyme and that only intravenous pepsinogen produced a rise in urinary pepsinogen; this confirmed the opinion of Gehrig (1886) that pepsinogen difused from the peptic cells to the blood where it circulated as pepsinogen and was excreted as such in the urine.

I have avoided a detailed analysis of the various papers published early in the century which purported to show an increase of urinary pepsinogen after feeding or injecting pepsin and the various theories put forward to explain these findings. They were based on crude methods of peptic assay and in the light of more modern methods the observations are invalid and the conclusions and theories deduced inadmissible on that score, and are only of limited historical interest.

THE RELATION BETWEEN GASTRIC ACTIVITY AND URINARY PEPSINOGEN.

The relation of urinary pepsinogen to gastric secretory activity has been and still is the most uncertain and confused in the whole subject of the physiology of pepsinogen. The confusions and contradictions of the earliest authors resulted only in part from their crude methods of peptic assay; these contradictions are still evident today, even with accurate methods. The key to the whole problem is the renal mechanism of pepsinogen excretion, and all that has been written on the subject of the relation between pepsin in the stomach and pepsinogen in the urine has to be re-examined and largely discarded on that score.

A review of the literature on the subject of this relation only serves to emphasize this understandable confusion, and is merely quoted, briefly to serve as an historical background.

Brücke (1861) and Sahli (1885) believed that the urinary enzyme was related to gastric contents, a view not held for very long. Some time later the clinicians tried to relate urinary pepsinogen to the secretory activity and the mass of stomach tissue destroyed or degenerated in carcinoma and "achylia". A few examples will show the contradictions - Wilenko (1908) found a decrease of urinary pepsinogen associated with a decreased gastric pepsin; Ellinger and Scholz (1910) stated that a low urinary pepsinogen with "achylia" was more in favour of carcinoma than of simple achylia; Teschendorf (1927) found that with carcinoma and depressed gastric secretion urine pepsinogen could be either normal, diminished or absent, and

Rothschild (1950) in confirming this finally stopped the whole controversy and stated that there was no relationship between urinary pepsinogen and gastric activity.

Gottlieb (1924) was one of the few authors who approached the subject with some semblance of logic. He correlated gastric acid and juice volume with serum pepsinogens and with urinary pepsinogen and showed that there was some positive correlation between hypersecretors of volume and increased urinary pepsinogen excretion. While recognizing that normal kidneys were necessary for accurate blood and urine levels, he did not realise that variations in renal function which occur normally and adjustments which occur constantly are the factors to be taken into account. Consequently his results are no more than suggestive of a positive correlation and this correlation is valueless.

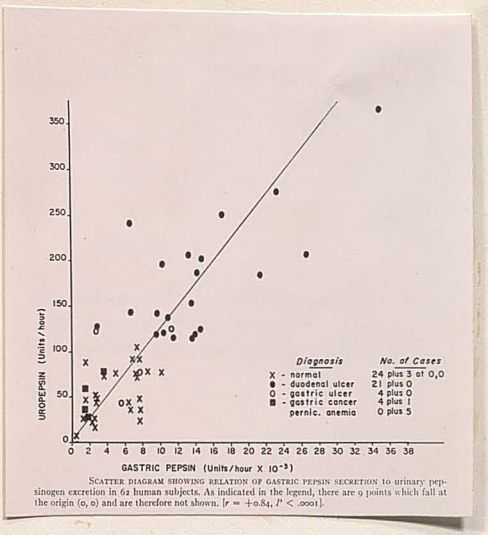
Three recent papers deal with this attempt to correlate directly urine pepsinogen with gastric function.

Broh-Kahn, Mirsky and Podore (1948) arguing a priori without actual simultaneous studies, concluded that the urinary pepsinogen reflects the intrinsic or endocrine activity of the peptic cells of the stomach and was independent of gastric juice stimulants, especially of food. Therefore the factors which cause an increase of urinary pepsinogen act independently on the endocrine secretion of these cells. They go on further to discuss the relationship with hyperacidity and found that though there was a frequent association, there is no causal relationship between the two factors and that

the increase of both in ulcers was fortuitous and may be ascribed to the influence of unknown factors which may act to increase either acid production in the stomach and/or urinary pepsinogen excretion. Mirsky (1950) stated that a very minute amount of pepsinogen was secreted from the stomach to the blood and that when there is an increased secretion into the stomach, there was also an increased urinary pepsinogen excretion.

The two reports of simultaneous gastric pepsin secretion and urinary pepsinogen excretion (Janowitz and Hollander 1951) and Varro, Faredin and Novaszel (1952) have diametrically opposite results.

Janowitz and Hollander (1951) studied 62 subjects comprising 21 with duodenal ulcers, 4 with gastric ulcers, 5 with pernicious anaemia, 5 with carcinoma of the stomach and 27 controls. All had been on liquid diet for 24 hours, fasting for another 16 hours. Simultaneous complete gastric emptying and total urine secretions were collected for 3 hours. They found a high positive correlation ($r = +0.84$, $t = 11.9$, $n = 60$, $p < .0001$) throughout the whole range of gastric secretion, valid for ulcers as well as non-ulcers. They estimate the proportion moving in both directions from the peptic cells to be constant at about 99:1. They conclude that urinary pepsinogen is a direct measure of gastric function. (See graph 17) As shown earlier (p.99) an attempt to repeat this work failed to corroborate these observations and explained why they were unlikely to be accurate.



SCATTER DIAGRAM SHOWING RELATION OF GASTRIC PEPSIN SECRETION to urinary pepsinogen excretion in 62 human subjects. As indicated in the legend, there are 9 points which fall at the origin (0, 0) and are therefore not shown. [$r = +0.84$, $P < .0001$].

Fig. 17. Correlation of gastric pepsin and urinary pepsinggen (Janowitz and Hollander 1951).

On the other hand, Varro et al (1952) studied simultaneous 12 hour secretion of gastric pepsin and urinary pepsinogen in 28 subjects (comprising 15 normals and 13 patients with ulcers. They found no positive correlation between gastric pepsin and urinary pepsinogen. In the ulcers the gastric pepsin was 3.25 times higher than the controls and the urinary pepsinogen 0.62 of that in the controls. They found that the gastric juice volume did not correlate either with urinary pepsinogen as Gottlieb (1924) had.

That, then, is the state of confusion which exists at present

and the knowledge of the relationship between gastric activity a nd urinary pepsinogen is no clearer than it was at the turn of the century.

Evidence presented earlier in this chapter (p. 105) explains these incongruities and contradictions.

One thing is certain - there can be no direct mathematical relation between gastric secretion and urinary pepsinogen that does not take into account the tubular reabsorption of pepsinogen. The estimation of urinary pepsinogen can have little direct bearing on the exact state of gastric secretion. That explains why so many normal levels of urinary excretion are found with high gastric pepsin; that explains why there are so many apparently inexplicable and unpredictable daily variations; that explains the lack of effect of gastric stimuli (it has been shown that the variations of urinary pepsinogen in response to gastric stimulation are the result of other effects of that stimulation and of the unnatural dehydration upon the renal clearance, p. 106); that explains why no direct correlation has been found, or is indeed possible between urinary pepsinogen and gastric acid, and confirms the statements of Broh-Kahn et al (1948) that the association is purely fortuitous and probably dependent on simultaneously acting outside factors.

Nevertheless, the estimation of urinary pepsinogen may serve to indicate trends in groups of patients as shown in the next

chapter.

If pepsinogen estimations are to play any part in medicine, however, it will be the estimation of plasma pepsinogen which is a more direct guide of gastric function, being independent of imponderable renal mechanisms.

THE EFFECTS OF GASTRIC STIMULATION

The effects of gastric stimulation on urinary pepsinogen, or rather the lack of effect found by many workers, should also be re-evaluated with the knowledge of the renal mechanisms of pepsinogen excretion, as one has to do wih the direct relationship between gastric secretion and pepsinogen in the urine. These observations are, after all, simply an extension of those in the foregoing section.

Once again one is faced with the mass of contradictory evidence on the effect of meals on urinary pepsinogen. Von Calcar (1910) (sham feeding) Pechstein (1911) Loeper and Debray (1922) and Gottlieb (1924) found a post-prandial rise ofurinary pepsinogen. Mirsky et al (1948) found by careful study that meals had no effect on urinary pepsinogen. These are but a few of the many reports with conflicting views. It appears, however, from more careful studies that meals have no effect on urinary pepsinogen.

With regard to gastric stimulation by caffein and by

histamine, Bucher and Anderson (1947) found that these stimulants had no effect on the urinary excretion of pepsinogen in cats; Gehrig (1886) found pilocarpine to have no effect and Peczenik (1927) that double vagotomy and sympathectomy had no effect.

As shwn from experimental evidence earlier in this chapter (p. 100) gastric stimulation by caffein and histamine had a definite effect on pepsinogen in the stomach, the blood and the urine. These effects, however, were not consistent - in some there was a definite large increase of gastric pepsin, acid and juice volume with a fall in plasma concentration of pepsinogen, a drop in urine pepsinogen caused by a decreased renal clearance of pepsinogen. In others, the renal clearance remained the same, as the urine pepsinogen. It seems, therefore, that any effect these stimulants might have on urinary pepsinogen will depend on the changes produced in plasma concentration and in renal clearance of pepsinogen, which will in turn partly depend on the amount of fluid lost by gastric secretion.

Once again, no direct consistent relationship between stimulated gastric secretion and urinary pepsinogen seems possible, unless one takes into account the secondary effects of those factors on renal mechanisms.

THE EFFECTS OF DIET AND STARVATION ON URINARY PEPSINOGEN.

The effects of changes in diet (as opposed to meals) and of starvation on urinary pepsinogen have been reported in a number of careful studies in man and in animals. These reports are an extension of a large number of reports on the effect of diet on gastric secretion, most of which agree that a high protein diet will progressively increase gastric pepsin (Gottlieb 1924; Delhougue 1927) though Smith and Cowgill(1933) found no increase of secretion in Pavlov pouch dogs under these conditions.

DIET

With regard to the effects of diet on urinary pepsinogen, I believe that Hoffman (1887) was the first to show an increase in urinary pepsinogen in response to a high protein diet. This was later confirmed in man by Gottlieb (1924) and by Bucher (1947) and in animals by Peczenik (1927) and Peczenik and Kawahara (1927).

The following table summarizes the evidence presented by Bucher (1947) in support of these findings - on 30 normal women students fed on 3 different diets for 2 days after control observations for 2 days on normal diet.

Group	24 hr. urine volume C.	E	pH urine C.	E.	pH digest C.	E	Dietary protein C.	E	Urine N_2 C.	E	Urine pepsinogen units/24 hours. C.	E.
High Protein 12 women	1191	1809	6.25	580	2.22	2.31	62	162	9.5	21	243	360
Milk 9 women	820	1774	6.09	6.27	2.22	2.05	76	97	8.7	12	206	204
Orange juice 9 women	1440	2018	6.21	7.45	2.10	2.21	62	27	10	5	400	159

C = 2 control days.

E = 2 experimental days.

TABLE NO: 30 EFFECT OF DIETARY PROTEIN ON URINARY PEPSINOGEN
(Bucher 1947)

Other rather interesting facts have been recorded. Loeper (1914) found that milk-feeding caused an increase of a urinary enzyme acting better on casein than on albumin; Pechstein (1911) found that infants fed on breast-milk excreted less urinary pepsinogen than infants fed on cows milk or artificial diets.

Peczenik and Kawahara (1927) found that in the frog only horse-serum would cause an increase in urinary pepsinogen - milk, meat, albumen, cow-thyroid and gastric mucosa had no effect; in guinea pigs carbohydrate caused an increase of urinary pepsinogen, but not milk, the reverse being true in the very young. Mice behaved exactly opposite to guinea pigs; in rats meat and fat cause an increase, but pure egg albumen and sugar cause a drop in urinary pepsinogen.

STARVATION

The effects of starvation were studied by Peczenik (1927). In man he found that 8 days starvation caused no drop in concentration of urine pepsinogen which fell to half in 16 days and was then restored to normal 2 hours after the first meal.

The effects of starvation in aminals were shown by Peczenik to depend on the diet before starvation. 3 days starvation after carbohydrate reduced urinary pepsinogen to less than half; 4 days starvation after 9 days meat diet produced no change. The rats were starved to death after various diets and showed no drop after protein feeding till just before they died from inanition. Fat

produced results midway between carbohydrate and protein. The same holds for the cat as for the rat, but in the guinea pig the reverse is true.

He found also that thyroid feeding caused a progressive rise in urinary pepsinogen, even in the fasting state.

Bucher and Ivy (1947) found that refusal of food by cats caused a diminution but not a disappearance and that in prolonged fasts there was a decreased total output of pepsinogen in man and animals. They comment that the concentration of enzyme may even rise due to oliguria.

This later statement is the main criticism one can lay against Peczenik's work, since all his results are expressed as concentrations of enzyme and not total amounts (see also p. 73)

Observations by Merten (1948) done during the World War II in Germany showed that in 1943-44 there was a general decline of urinary pepsinogen duento food shortage. In malnutrition oedema, pepsinogen is absent from the urine.

It appears agreed, therefore, that starvation will cause a decrease in pepsinogen in the urine and protein feeding some increase, independent of the actual meals. Whether either of these conditions produces the results by action on renal function or by primary action on the stomach, would be difficult to say without careful investigation.

THE RELATION OF URINARY PEPSINOGEN TO RENAL FUNCTION

As has ben shown earlier in this chapter (p. 150 et seq.) pepsinogen is reabsorbed to the extent of 60 to 98% in the proximal tubules after being filtered through the glomeruli, and various conditions may exert an influence on the concentration or rate of excretion of th urinary enzyme. These have been discussed in that section.

However, certain facts may best be dealt with in this section since they relate more particularly to pepsinogen as it is found in the urine; these are the relation of urine pepsinogen to urine volume and concentration, to the pH of the urine and to chloride excretion in the urine.

(a) THE RELATION BETWEEN PEPSINOGEN AND THE VOLUME AND CONCENTRATION OF THE URINE.

The importance of this connection lies mainly in the evaluation of published results of urinary pepsinogen estimations. Before a truly quantitatiye method came into use, it was possible only to estimate the concentration of pepsinogen in the urine - this was done by the digestion of solid media (Mett's method) or by the dilution technique (see Chapter II). In order to do sc, these workers, relying on crude methods, had to use the most concentrated urines which contained the maximum concentration of pepsinogen. The morning specimen of urine was found to be the most concentrated and most results are given as concentrations of pepsinogen in morning urines. These results are only of value in detecting the absence

of pepsinogen but not in assessing true differences of pepsinogen excretion.

Wilenko (1908) first stressed that the estimation of rate of excretion was a more accurate way of determining the excretion of pepsinogen than the concentration. This was later stressed again by Gottlieb (1924) and by Bucher (1947). Mirsky et al (1948) paid special attention to this problem and studied the daily excretion of 13 patients from 11 to 190 days and showed that the enzyme excretion does not depend on normal variations in urine volume. "Variations in urine volume in the overwhelming majority of subjects play no significant part in the regulation of uropepsin excretion." However, as shown previously forced diuresis may increase the urinary excretion of pepsinogen (p. 157) and dehydration may diminish the excretion.

It was felt that the necessity for estimating pepsinogen excretion as a rate per unit time rather than as concentration has been so adequately proved by the work of Mirsky et al (1948) that no further analysis of my material on this subject was necessary.

(b) RELATION BETWEEN URINARY PEPSINOGEN AND THE pH OF THE URINE

It has been proved that the urinary proteolytic enzyme under discussion is only pepsinogen (p. 166) and not a mixture of pepsin and pepsinogn, as supposed by Ellinger and Scholz (1910). The stability of pepsinogen in alkaline urine was in fact one of the first pointers to its identity (Gehrig 1886) and the stability was shown by Bucher (1947) (see p.167).

The independence of urinary pepsinogen of urine pH has been shown by Gottlieb (1924) by Farnsworth et al (1946), by Bucher (1947) and by Mirsky et al (1948).

Bucher (1947) analyzed the frequency distribution in 139 urines of pepsinogen and pH and found that the urines with the highest pepsinogen had the lowest pH. Incidentally this was the group which had had a high protein diet and it is probably the protein which accounts for the more acid pH rather than the low pH accounting for the high pepsinogen - in fact, a chance association.

Once more, no analysis will be made of the data in this study relating to this subject, since previous work is quite clear and unequivocal in denying any relation between urine pH and pepsinogen.

(c) RELATION BETWEEN URINARY PEPSINOGEN AND CHLORIDE EXCRETION

Experimental observations.

In order to examine any possible relation between chloride excretion and pepsinogen excretion, the two were determined in 104 specimens of urine from 34 consecutive patients studied. As shown in the following table, no constant association between urine chloride, pepsinogen or volume was detected in these 104 urines from 14 controls, 11 gastric ulcers and 9 duodenal ulcers.

GROUP	No. of cases.	No. of Urines.	Volume of urine ml./hr.	Pepsinogen P.U.%	Pepsinogen P.U./Hr.	Chloride mgm.%	Chloride mgm./hr.
D.U.	9	28	66.8	125	848	570	380
G.U.	11	35	65	120	780	492	320
Control	14	51	52.3	64.5	340	660	345

TABLE NO: 31 RELATION BETWEEN URINE VOLUME, PEPSINOGEN AND CHLORIDE IN 104 URINES.

The only relation between urinary pepsinogen and chloride is an inverse one and has been found post-operatively in partial gastrectomy where salt is retained and pepsinogen not. After 5 days or so, both return to normal levels (see p. 195). This is probably simply due to altered renal dynamics resulting from changes after gastrectomy superimposed on altered gastric production of pepsinogen, and not to a stress effect.

PATTERN AND VARIATIONS OF URINARY PEPSINOGEN EXCRETION

Early work on the subject shows no reference to any study of individual variations from day to day or from specimen to specimen, although these same authors attempted to show variations in relation to time of day, to food and other variabled.

It was not until recently that Broh-Kahn et al (1948) investigated the problem statistically. The first problem they investigated was the diurnal variation of which Gottlieb(1924) was convinced and which Bucher (1947) followed. They found that there was no significant difference between day and night urines. They collected on successive days urines varying up to three months in some sleeping versus waking urines. They found that the amount excreted per day varied from day to day but as mentioned above, diurnal variation was not significant and analysis by distribution frequency from night urines applied (a) on a percentage cumulative frequency against pnits of variables on probit paper and (b) logarithmic units against probits, suggested that both produced a straight line, whereas the plotting of arithmetic units against probits did not produce a straight line. They therefore go further than simply expressing their results as total amounts and recommend that they should be expressed as log units per unit of time.

The next point investigated by Broh Kahn et al (1948) was whether there was a constancy of formation of urinary pepsinogen throughout the 24 hours, on which depended the validity of expressing excretion as a rate per unit of time. This constancy was measured

in two ways, (a) comparing in the same subject night versus day urines for several consecutive days in 5 subjects, chosen at random from 9 to 53 days (average 31 days). The "t" test applied to this investigation did not even approach significance in 4 cases at a 5% level, this being independent of the subjects' characteristic level of excretion which varied from 0 to 1.01 log units per hour, that is, throughout the whole range of normal excretion.

The next point investigated was whether the duration of collection confirmed the constancy of formation of urinary pepsinogen throughout the 24 hours, since the average waking day consists of 16 hours and the average sleeping night of 8 hours. Urines were then collected from 2 to 24 hours and no effect of duration of collection was found, and they conclude "that fluctuations in uropepsin excretion per hour are not affected appreciably by the time of day during which the urine is collected or by the duration of the collection period." Therefore it is practical and permissible to collect only night urines to represent the true 24 hour excretion.

Despite this evidence, Broh Kahn et al found, as I have, that there may be sometimes fairly large fluctuations of excretion from day to day. 695 urines from 27 healthy men from 1 to 127 days were analysed both by averages and by an analysis of variance to determine the number of specimens needed to overcome statistically this fluctuation from day to day. They conclude that since the mean variance within the subjects in the rate of "uropepsin"

(0.0474) is smaller than the mean variance among all the subjects (0.0929)(the ratio of these two variances is greater (F = 1.96) than would be expected by chance in 95/100) that most differ significantly from each other in the rate of "uropepsin" excretion and that there is no first or subsequent sampling effect. They also compared 4 against 10 samples in individuals in whom 10 or more samples were estimated and show that the variation in the individuals was less than between the individuals.

They finally conclude that the pattern of pepsinogen excretion is constant for healthy men from day to day, secondly that small fractional samples during the day are representational (S.D. ± 62%) for the individual.

In discussing the cause of this constancy they remark that it is independent of sleep or wakefulness, work, exertion, recreation, food or meals, seasons, rate of urine formation and the time of day. They further point out that with the lack of correlation with gastric juice that they believe that different factors control the endocrine and exocrine pattern of pepsinogen metabolism, and finally, that no single factor had been discovered which controls this pattern of urinary pepsinogen excretion.

Other work on the relative constancy on successive days has confirmed or rather preceded the work of Broh Kahn et al (1948) and that was the simple results of Bucher (1947) and Gottlieb (1924) the former finding relative constancy from day to day and the latter relative constancy from month to month.

In summary, therefore, Gottlieb (1924) showed the relative constancy in the individual from day to day of total urinary pepsinogen excretion and Bucher (1947) confirmed these findings and extended them to observations which showed constancy from month to month. They believed that there was a diurnal variation.

Broh-Kahn et al (1948) investigated individual patterns of excretion and found that though there was some daily variation that there was no significant variation during the day and that any variations found could not be causally related to sleep, waking, activity, recreation, food and meals, the time of day or the pH and volume of the urine, and if dependent on any one other thing, that factor was unknown and was not responsible for the fluctuations of secretion of the stomach into its lumen.

The factor which they do not consider and which obviously plays a major part in regulation of urinary pepsinogen excretion is the kidney, but whether it does so in response to one controlling factor, such as adrenal or posterior pituitary; or whether it does so simply as an incidental to its major work of regulating the milieu interieur, is not quite clear. To believe the former one would have to postulate a physiological significance to the endocrine secretion of pepsinogen, and I admit one is unable to do so.

NORMAL LEVELS AND UNITS

A factor which has been responsible for the retardation of progress in the study of urinary pepsinogen has been the diversity of units used resulting from the lack of uniformity of methods employed.

Prior to the use of the haemoglobin method of peptic assay (Anson and Mirsky 1932) and its use for urinary peptic assay (Bucher 1947) many different methods were employed. The earliest investigations simply estimated the concentration of enzyme in the early morning specimen and sometimes even compared this to a "control" urine as a standard. Gottlieb (1924) alone, attempted to do away with pseudo-quantitative studies and set up as a standard Armour scale pepsin. He expressed results in equivalent peptic power to that / of pepsin. This was an admirable and very sound piece of work, though one cannot be certain that the Armour pepsin was always standard.

However, the introduction of a comparative technique (haemoglobin) (see p. 56) did away with the need for the tedious comparison with "standard" pepsin and the necessity for depending on a commercial firm to produce an any more reproducible standard by the universal methods in use for peptic assay.

It seems a pity that since a standard comparative method capable of reproducibility under varying conditions has been introduced, that a standard form of reporting results has not been

adopted. This problem is discussed in detail in the chapter on method (see p. 78).

The only agreement reached has been the expression of results in units (whether mgm. tyrosine, or mEq. tyrosine or log units) per unit of time (1, 12, or 24 hours).

EFFECTS OF AGE AND SEX ON URINARY PEPSINOGEN

The only systematic work on pepsinogen excretion in infancy was done by Pechstein (1911) who determined the concentration of pepsinogen in the urine by the edestin method (see p. 44). He found that the average concentration in the infant was 1/20th that of the adult. In 3 infants on the first day of life he found pepsinogen present in the urine as well as rennet ("labzymogen"). This rises rapidly to a level which remains constant for the first year and is higher in artificially fed than in breast-fed children.

Farnsworth et al (1946) who also determined only the concentration of enzyme found some decline in old age and Bucher (1947) believed that this was suggestive but not in any way definite. She appears to argue more from the known decline in old age of the gastric hydrochloric acid secretion.

In this study comprising 36 males and 24 females no significant differences relating either to age or to sex could be detected (pp. 217 ff). There was also no relation between pepsinogen excretion and age in the ulcer patients, though sex differences were marked in the pyloro-duodenal ulcer group (p. 227). Normal control levels may be taken as 194 S.D. ± 132 P.U./Hr for

males and 244 S.D. ± 154 P.U./hr. for the females.

SECTION VIII

THE RELATION TO STRESS.

A. MENTAL STRESS

Only one paper on the relation between urinary pepsinogen and mental stress has appeared, Mirsky, Caplan and Broh-Kahn (1949) who studied the night urines from 6 subjects under psychiatric observation of up to 2 years. 3 of the 6 were having psychotherapy. They also studied 18 so-called normal men as controls who kept a diary of events and emotions. Other material used was from other psychotherapists without prior knowledge of the case material. A few cases are discussed in detail and are in summary as follows:

Case (1). A 28 year old male, apparently normal but with many compulsive traits whose excretion was steady for 36 days, then showed a 20 day marked increase after a decision tohhave a child and the discontinuance of the use of contraceptives. No obvious anxiety or tension could be detected by the ordinary anamnestic approach.

Case (2). A 29 year old male showed a rise for 10 days following a similar experience as that of case (1), a 5 day rise following the positive Friedmann test and a third rise at the time of the child's birth.

Case (3). was a 28 year old male who showed several rises in response to resentment, anger, the pleasure of an official engagement and subsequent frustration with his prospective parents in law.

Case (4) was a 30 year old female with a duodenal ulcer who apparently excreted an increased amount of pepsinogen and showed further rises when faced with a jealousy situation comparable to her unhappy childhood.

Case (5) was a 44 year old man who was the repressed son of a dominant father who finally broke loose under psychotherapy but with such guilty complexes that he developed symptoms and a haematemesis paralleled by an increase in urinary pepsinogen excretion.

Other cases are quoted in whom values similar to peptic ulcer values were found in a few controls who were then found to have an oral-receptive personality, without conflict however. One such patient, 4 months after an increase of urinary pepsinogen, developed a peptic ulcer which disappeared on treatment but with the persistence of high urinary pepsinogen. Mirsky concludes that it is not meant as a pointer to the aetiology of ulcer but an increase of the urinary pepsinogen resulted from a situation threatening or mobilising the individual's unconscious wish for love and being taken care of and was notthe conscious knowledge of conflict. He recommends that this method should be used in the long term study of the relation of the psyche to gastric dysfunction. Mirsky goes on further in the discussion

to say that "dramatic situations such as those produced by surgical operations always produce a marked rise in urinary pepsinogen, also A.C.T.H. Consequently it is possible that any stimulus which is sufficiently stressful may produce an increase of uropepsin by the stomach and an increased urinary excretion, presumably as a result of hypothalamic and pituitary stimulus." One small point added to this question of mental stress is that in a group of females studied by Bucher (1947) there was an increase of urinary pepsinogen on 2 days, 3 weeks apart, during the control period, which occurred at the time of examinations. She wrongly, I believe, attributes this to lack of sleep since it has been shown that wakefulness does not influence urinary pepsinogen, and one must assume that this was not due to lack of sleep, but due to the mental stress of impending examinations. One cannot argue with the factual evidence presented by Mirsky, Caplan and Broh-Kahn (1950), nor with the conclusions that it was an unknown psycho-endocrine set of conditions which was reflected by urinary pepsinogen changes.

The next section will show that the statements by Mirsky of an increase of excretion after operations does in fact occur, but is limited to partial gastrectomy and that the increase mentioned occurring after A.C.T.H. is not in fact a universal experience. Nevertheless, the oral-receptive dependent personality as shown by Mirsky and the fact that it is an unconscious conflict which produces these changes is worthy of further investigation along these lines. In one subject whom I investigated from this point of view, 4 observations

during a non-stress period were 26, 0, 144 and sometime later 87. Shortly after that, undergoing a stress period related to examinations, the subject's output in the day awaiting results was 256 units/hour. This difference is within the normal range of fluctuation. Nevertheless, this is only one observation and cannot be used to comment on Mirsky's observations and conclusions.

EXCRETION OF PEPSINOGEN IN THE URINE FOLLOWING OPERATIONS.

Investigations into the source of the urinary enzyme led one to the estimation of urinary and in some instances blood pepsinogen following operations on the stomach. Previous workers had shown the disappearance of pepsinogen from the urine after total gastrectomy in dogs (Frouin 1904, Matthes [redacted] 1903, and Von Calcar 1910); in cats by Bucher and Ivy (1947) and by Balfour, Preston and Bollman (1948) in rats, and the mention of this fact in humans by various other authors (Bucher 1947, Mirsky et al 1948, and Janowitz, Levy and Hollander 1950).

Experimental observations.

I found a persistence of a small amount of pepsinogen in the urine after total gastrectomy in some cases, the levels never exceeding 100 units/hour, but in some the urine was completely free of pepsinogen. This would suggest that what little pepsinogen is found in the urine in these circumstances probably arises from the leucocytes, since the plasma is free of pepsinogen (p. 124).

Going on further to investigate the effect of partial removal of the stomach, interesting and surprising results were found. In both lesser-curve and prepyloric gastric and in duodenal or stomal ulcers, partial gastrectomy either Polya-Hoffmeister or Bilroth, there was a great increase of pepsinogen in the urine for the first 3 or 4 days after the operation, reaching in some instances levels not seen at any other time (see table). This study comprised 15 patients with ulcers (3 female, 12 male) and 1 female with carcinoma. The post-operative rise could not be correlated in any way with the sex of the patient, the site, the duration of the ulcer, or the type of partial gastrectomy. Of the 16 peptic ulcers, 15 had a partial gastrectomy, one a sutured perforation; of these 12 had a distinct rise in pepsinogen excretion, 3 a decrease (of these one died on the second post-operative day) and one showed no change. The one patient with a prepyloric carcinoma who had a subtotal partial gastrectomy showed a decrease for two days and a return to her highest pre-operative level on the third day. (see also graph. fig 18.)

OPERATION	GROUP	NO. OF CASES	PREOP. MEAN	Dayse before op. 3	2	1		Days after operation. 1	2	3	4	5	6	7	8	9	10
Various	Control	8	228	117	250	266	NITROGEN	153	185	101	191	171	-	-	-	-	-
P.Gastr.	G.U.	6	635	534	799	557	AMINO	919	1375	959	501	118	228	-	174	-	413
P.Gastr.	D.U.	10	674	732	704	630	PEPTONE	1038	1069	1070	629	332	165	142	671	486	293
Total Gastr.	Ca.	3	207	120	330	170	NITROGEN	62	41	-	-	75	0	62	-	-	-

TABLE NO: 32 EFFECT OF OPERATION - PARTIAL AND TOTAL GASTRECTOMY AND VARIOUS NON-GASTRIC OPERATIONS - 27 CASES. (See also graph 18).

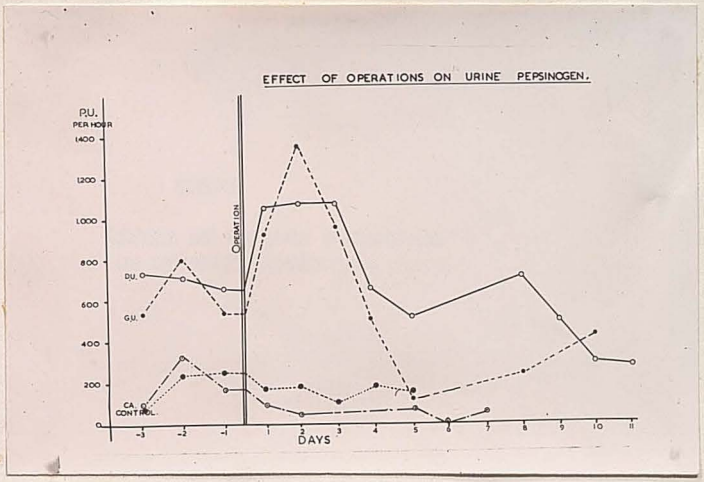


Fig. 18. Effects of various operations on urinary pepsinogen excretion (see text and Table No. 32)

In order to explain this great increase, one of three mechanisms could be operative - either the manipulation of the stomach mechanically expelled pepsinogen into the circulation or there was some post-operative diminution in the reabsorption of pepsinogen in the tubules of the kidney. Thirdly, handling the stomach might cause irritability of the stomach with hypersecretion of the remaining fundal portion. Finally, all three mechanisms might operate in causing this phenomenon.

To investigate these hypotheses, several experiments were done.

I. In the first, the effect of total gastrectomy on plasma pepsinogen was studied in three patients with carcinoma of the stomach.

In the first patient, (A.B.) plasma pepsinogen at the start of the operation was 20.3 P.U.%; at the end 0. Simultaneously with the first, blood from the gastric vein contained 46 P.U.%.

In another (D.C.) the pre-operative plasma level was 16 P.U.%; 2 hours after operation 12 P.U.% and 20 hours after operation 6.1 P.U.%. 1 week after the operation - 0.

In a third patient the pre-operative level of plasma pepsinogen was 19 P.U.% which fell to 0, 24 hours after operation and remained so for the next 5 days.

These figures suggest that no great amount of enzyme is extruded into the circulation by the handling of the stomach during the operation, and that increased blood levels during the first few days following partial gastrectomy (see below) depends on the continued secretion by the fundal remnant of the stomach. It further suggests that the stomach secretes pepsinogen continuously (or rather that there is a continuous diffusion of pepsinogen from the stomach) into the blood stream, and that it takes from 2 to 48 hours to excrete what there is in the blood into the urine. If one assumes the extracellular volume of fluid in the body to be 15 litres and the average concentration of pepsinogen in this fluid to be 20 P.U. per 100 ml. (pepsinogen is diffused equally throughout the body - see p. 136) making a total content of ± 3,000 P.U. in the extracellular fluid of the body.

The excretion of this amount after total removal of the gastric source, at say 200 P.U. per hour should take no more than 24 hours. What re-appears in the urine several days after total gastrectomy could be due to some of the pepsinogen from the leucocytes being excreted.

II. In a patient with a duodenal ulcer (W.T.) the blood and urine pepsinogen were estimated before and after partial gastrectomy and the renal clearance of pepsinogen (CSp) calculated from these figures. These figures are:-

Urine	Days before operation.				Days after operation.				
	4	3	1		1	2	3	4	5
Urine pepsinogen (P.U.per hr.)	300	400	327	OPERATION	760	1510	2460	1220	640
Plasma pepsinogen (P.U.%)	28	26	21		26	63	61	31	47
CSp ml./minute	17.7	28.6	17.4		48	36.5	67.2	66	22.6

TABLE NO: 33 THE EFFECTS OF PARTIAL GASTRECTOMY (see text and graph)

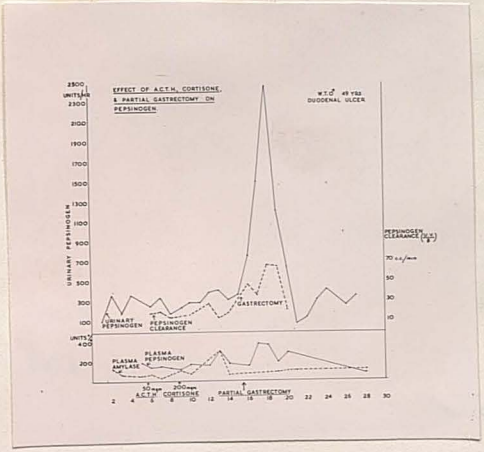


Fig. 19. Effects of A.C.T.H., Cortisone and partial gastrectomy in one patient (W.T.) on urine pepsinogen pepsinogen clearance, plasma pepsinogen and amylase.

These figures clearly show that blood and urinary levels and clearance figures of pepsinogen by the kidney are fairly constant preoperatively, whereas postoperatively on the first day there is no increase of plasma pepsinogen, but withal an increase of urinary pepsinogen and clearance to approximately double pre-operative levels, and on the next four days an enormous increase in urine pepsinogen, some increase in plasma pepsinogen and a great increase in renal clearance of the enzyme.

These figures seem to indicate that the handling of the stomach during operations does not cause any increase in plasma levels or account for any increase in urine levels - further confirming the studies on total gastrectomy. The increase of plasma levels in the next four days has to be explained by an increase of diffusion from the stomach - possibly by reactive hyperaemia and an alteration of the gastric haemodynamics since none is being held back by the kidney - infact, the reverse occurs.

We have to look elsewhere, therefore, for the explanation of this increase in urinary pepsinogen following partial gastrectomy. The increase of the urinary enzyme is out of proportion to the increase in plasma level and this can be explained by the increase in renal clearance of pepsinogen. Elsewhere it has been shown that from 60 - 98% of pepsinogen, which is freely filtered through the glomerulus, is reabsorbed in the tubules. The increase in clearance, therefore, indicates that less is being reabsorbed in the tubules and therefore appearing in the urine. Whether this is due to altered renal haemodynamics or to the effect of adrenal steroids on the renal mechanism is difficult to say.

That this phenomenon depends on the actual surgery of the stomach as well as post-operative renal changes follow from the next set of observations on non-gastric operations.

III. Having found the increase of pepsinogen diffusion and secretion in partial gastrectomy, the next step was obviously, to see whether the same increase occurs with operations other than gastrectomy. The urinary pepsinogen excretion was determined in 8 patients in whom the operations were cholecystectomies on 2, splenectomy, meniscectomy, arthrodesis of hip, amputation of an arm, excision of Dupuytrons contracture scar and the insertion of a tibial traction pin respectively on the other 6. All were performed under general anaesthesia.

As will be seen from the graph and tables the overall tendency was a fall from the pre-operative levels and in no instance did the post-operative levels rise above 422 P.U./hr. and all except one were below 310 P.U./hr. At least half these operations were as long and traumatically as severe as partial gastrectomy. One therefore has to assume that the essential component of the group of factors causing the rise of excretion of pepsinogen after partial gastrectomy is the presence of the fundal remnant of the stomach which has been irritated by handling and in which there is an increased blood flow for several days. The renal factor might be secondary to an abnormally high level of plasma pepsinogen together with some change in reabsorptive capacity for pepsinogen caused by the operation. Expression of pepsinogen from the stomach by handling has already been excluded, as explained above.

SECTION IX

THE ADRENAL AND PEPSINOGEN

A number of recent reports by Gray, Benson, Spiro and Reifenstein (1950a, 1950b, 1951a, 1951b) has dealt with investigations into the effect of adrenocortical hormones on gastric secretion and urinary pepsinogen excretion. They showed that A.C.T.H. (with a healthy adrenal) and Cortisone increased gastric secretion and urinary pepsinogen often to levels seen only in some duodenal ulcers. They reported one case of Addison's disease who excreted no pepsinogen and who excreted a normal amount in response to cortisone, but not in response to A.C.T.H. They showed that a pernicious anaemia and a total gastrectomy excreted no pepsinogen before or after A.C.T.H. or cortisone.

They state that A.C.T.H. 100 mg./day will produce a large response within 24 - 36 hours and a return to normal within 7 days. All their cases showed this large response, averaging an increase of 126%. The same response was found in gastric pepsin, acid and volume. The dosage of A.C.T.H. was from 200 to 1070 mg. given over 4 to 21 days in 10 cases; an 11th case received 2770 mg. over 36 days.

With cortisone the response was slower; it was also delayed in patients with peptic ulcers.

This work was partly repeated in this study and their results could not be confirmed.

RESULTS.

Owing to the shortage of A.C.T.H. and cortisone in England at the time this study was undertaken, this study is not as complete or as comprehensive as would be desired. The Medical Research Council kindly supplied me with 1.0 G. of A.C.T.H. and 3.0 G. of cortisone acetate for this purpose.

A. PITUITARY-ADRENAL INSUFFICIENCY

It was believed that Gray et al (1950 a. and b.) drew rather sweeping conclusions from one case of Addison's disease; accordingly, several cases of each of Addison's and of Simmond's disease were investigated. Some were having cortisone, as indicated below.

	BEFORE CORTISONE P.U./Hr.	DOSE OF CORTISONE mg. and days	AFTER CORTISONE P.U./Hrs.
Addison's Disease.			
Case 1.	175	-	-
Case 2.	-	100 daily x 65	213
Case 3.	258	100 once	511
Case 4.	71	100 b.d. x 2	280 210
Simmond's Disease.			
Case 1.	300	100 once	530
Case 2.	144	-	-

TABLE NO: 34 URINARY PEPSINOGEN IN PITUITARY-ADRENAL INSUFFICIENCY.

From these results (Table No. 34) it will be seen that 3 untreated cases of Addison's and 2 of Simmond's disease excreted normal amounts of pepsinogen and that cortisone administered to 2 of these patients produced a moderate increase of excretion, not inconsistent with the simultaneous increase of urinary volume. 2 cases on maintenance doses were excreting normal amounts.

B. THE EFFECTS OF A.C.T.H. ON URINARY PEPSINOGEN.

The effects of A.C.T.H. were studied on 5 cases; 2 had one dose (25 and 50 mg. respectively) and 3 had prolonged courses.

Case	OBSERVATION DAYS 1	2	3	A.C.T.H. Dose (mg)	Dayse.	DAYS AFTER START OF A.C.T.H 1	2	3	4	5	6
1.	725	322	758	25	Once	136	146	611	685		
2.	355	190	355	50	Once	260	345	187	251		
3.	316	23	15	100	15	-	-	-	-	-	-
						0	0 (Days 15 and 16)				
4.	256	42	260	75	7	492	240	225	87	233	202
5.	44	28	0	60	7	-	-	415	686	345	-
						195 (6 months)					

TABLE NO: 35 EFFECT OF A.C.T.H. ON URINARY PEPSINOGEN

Table No. 35 shows that of the 5 cases, only one responded by an increase in urinary pepsinogen, while 2 showed a marked drop and 2, no change. There is certainly not the uncanny consistency shown in the papers of Gray et al.

C. THE EFFECTS OF CORTISONE ON URINARY PEPSINOGEN.

5 cases were studied - they received doses from 50 to 1400 mg. Cortisone in from 1 to 20 days.

Case	DAYS BEFORE 1	2	3	CORTISONE Dose/ day	Days	DAYS AFTER 1	2	3	4	5	6	7
1	634	690	693	200mg.	7	-	-	-	653	625	442	
2	570	332	696	50mg.	20	----12.6		27	292(Days 16,17,18)			
3	355	260	345	200mg.	1	251	300	300	400	420	327	
4	230	318	145	100mg	5	160	250	46	100	184	252	260
5	-	331	420	200mg.	7	298	200	340	59	258	274	200

TABLE NO: 36 THE EFFECT OF CORTISONE ON URINARY PEPSINOGEN

The 5 cases shown in Table 36 above are different from the cases of Addison's and Simmond's disease mentioned earlier. Case 2 in Table 35 and 3 in Table 36 was a duodenal ulcer; the others had neither endocrine nor gastric disease and can be taken as normal controls.

Table No: 36 shows that of the 5 cases, the only one to show a response of any kind to cortisone, responded by a dramatic drop on the first days measured (16th to 18th). In case 4 of Table No. 35 and case 5 of Table No. 36 there was a temporary increase of urinary pepsinogen on the first two days after stopping the drug, paralleling a marked diuresis and accounted for by an increased pepsinogen clearance (i.e. diminished tubular reabsorption).

D. THE EFFECT OF A.C.T.H. AND CORTISONE ON PLASMA PEPSINOGEN

4 cases were studied to observe the effects of adrenal steroids on plasma pepsinogen, simultaneously with urinary pepsinogen. One ptient was given 50 mg. A.C.T.H. and three days later 200 mg. of cortisone by intramuscular injection. Another had 75 mg. A.C.T.H. daily by injection for 7 days; the third had 100 mg. cortisone daily for 5 days and the fourth 200 mg. cortisone a day for 7 days. In no instance was there any consistent change of plasma pepsinogen.

E. THE EFFECT OF A.C.T.H. AND CORTISONE ON RENAL CLEARANCE OF PEPSINOGEN.

In the 4 cases mentioned in paragraph D. above, neither hormone produced any effect on the clearance of pepsinogen, except in the second and fourth cases who showed a diuretic response in the first two days after discontinuing the drugs with a simultaneous increase in urinary pepsinogen and renal clearance.

SUMMARY

Contrary to the results of Gray et al (1950, 1951,) I have been completely unable to produce a consistent rise with either A.C.T.H. or cortisone; pepsinogen has been present in cases of Addison's and Simmond's disease. In fact in some cases exactly the opposite results have been found, i.e. a sharp drop in urinary pepsinogen. There is no consistent effect on the plasma pepsinogen.

One is forced to conclude that Gray et al were too keen to try and substantiate a theory and that their results are too good to be true.

CHAPTER V.

DISTURBANCES OF PEPSINOGEN METABOLISM IN DISEASE.

As with many other body constituents, pepsinogen was discovered and investigated as a physiological substance for some time before its possible application in clinical medicine became recognised, whereupon the physiological aspect was submerged in a mass of investigation into its clinical possibilities, appearing only as a by-product of that period of research.

From the first discovery of a pepsin-like substance in the urine by Brücke in 1861 and the prediction of the existence of pepsinogen as a precursor of pepsin by Schiff (1877) it was a small step to its positive identification by Langley in 1881 in the gastric mucosa. By then it was suggested that the enzyme was secreted directly into the blood (Gehrig 1886) and not reabsorbed from the gastro-intestinal lumen as supposed by Brücke.

The subsequent investigation into the clinical uses of urinary pepsinogen estimations produced a spate of papers

from the German clinicians of the late 19th and early 20th centuries which did little to elucidate the physiology and ended in confusion as to its true value in clinical medicine, a state somewhat rescued by the excellent work of Gottlieb (1924).

The discovery of the haemoglobin method of pepsin assay by Anson and Mirsky (1932) and the excellent work of Northrop (1919 et seq.) in establishing comparable methods of determination of peptic activity merely served to emphasize that the earlier German workers had failed, not because of lack of originality or of thoroughness but because of the inadequacy of their methods, an all too common cause of failure in many fields of research.

The isolation of crystalline pepsinogen by Herriot (1938) and the subsequent physiological studies of Bucher and her co-workers (1946 - 1947) and of Mirsky et al (1948), placed the study of this substance on a sound scientific basis and paved the way for the closer investigation into the disturbances of pepsinogen excretion in the urine by Mirsky and his co-workers (1948) and by Janowitz, Levy and Hollander (1950, 1951).

In the period before Roentgen diagnosis of gastric lesions became universal, carcinoma of the stomach was the major diagnostic problem of the gastro-enterologist. It is not surprising, therefore, that the bulk of the literature dealing with urinary

pepsinogen between 1885 and 1930 should be concerned with this problem. Since the results cannot be accepted in any quantitative way by modern standards, it would serve little purpose to review this work in detail. A few references will show how confused the results and conclusions were. In 1893 Van Noorden stated that in histamine fast achlorhydria, little urinary pepsinogen spoke for carcinoma, Fuld and Hirayama (1912) that in carcinoma there is a definite decrease of pepsinogen, Ellinger and Scholz (1910) differentiated simple achylias from carcinoma achylia and that a normal urinary pepsinogen spoke for carcinoma; they tried to go further and devised a scheme of prognosis depending on the proportion between gastric and urinary pepsinogen. Takeda (1910) agreed with these results; Bieling (1911) found that in carcinoma the urinary pepsinogen was usually absent and asserted that the quantitative estimation was important in the early diagnosis of carcinoma. As late as 1927 Teschendorf was still trying to differentiate achylia of carcinoma (where urinary pepsinogen is depressed) from true achylia where urinary pepsinogen is normal, but as did Rothschild (1930) concluded that the estimation of urinary pepsinogen had no value in the diagnosis of carcinoma or in the differentiation of achylias and the latter paper seemed to close the chapter of attempting the use of pepsinogen in clinical medicine until the next paper on the subject 16 years later when Farnsworth, Speer and Alt (1946) reported their results in the investigation of pernicious anaemia, and Bucher(1947) reviewed the literature and described some basic experiments.

Investigation of urinary pepsinogen excretion in relation to gastro-duodenal ulceration is relatively recent. The work of Mirsky and his collaborators at Philadelphia published in 1948 served to place this study on a sound footing. They established the relative stability of urinary pepsinogen excretion rates in normal men, and helped to establish the identity of the urinary enzyme as pepsinogen by feeding and injection methods (see Chapter IV), the techniques of estimation and more recently (Mirsky et al 1952) the same for pepsinogen in the blood. They then determined the disturbances of excretion of pepsinogen in gastric and duodenal ulceration, finding an increase over normal which was statistically significant. These studies were followed by those of Hanowitz Levy and Hollander (1950) who determined the relation between urinary pepsinogen and gastric pepsin, and then found that duodenal ulcers excrete about four times normal pepsinogen as controls and that gastric ulcers fall in the normal range.

Mirsky et al (1948c) found in the normal group that 98% of cases excreted less than 0.88 log units per hour, and 51% of all ulcers excreted more; in normals less than 8% excrete more than 0.78 log units per hour, whereas 50% of ulcers excrete more; 50% of normals excrete less than 0.47 log units per hour, whereas only 8% of ulcers excrete less than 0.47 log units per hour. It is therefore only of limited practical value in differentiating normals from ulcers, but nevertheless statistically quite clearly significant.

Janowitz, Levy and Hollander (1950) found less overlap between duodenal ulcers and controls. The following table summarizes their results.

	No.	RANGE U/Hr.	MEAN
CONTROL	44	0 - 136	47
D.U. or S.U.	35	114 - 373	180
G.U.	5	43 - 125	53

TABLE NO: 37 URINARY PEPSINOGEN EXCRETION (Janowitz et al 1950).

Varro, Faredin and Novaszel (1952) in a paper that is not very convincing, compare 18 ulcers with 12 controls, and find the following figures:

	RANGE	AVERAGE
12 controls	60 - 281	147
18 ulcers	125 - 421	268

TABLE NO: 38 URINARY PEPSINOGEN EXCRETION (Summarized from Varro et al 1952)

and conclude that these figures show no significant difference. They then go on to determine the "pepsin secretion quotient" by dividing gastric pepsin by urinary pepsinogen and

find the "quotient" higher in ulcers than in normals - which is simply another way of saying that gastric pepsin is higher in ulcers than in controls.

Other references to urinary pepsinogen excretion in ulcers are merely in passing in papers dealing with other aspects of the subject.

No-one has claimed, or can claim, that the estimation of urinary pepsinogen is an absolute way of distinguishing ulcers from normals and the statements made 63 years ago by Benderskey are as true today (and perhaps more so today) as when he made them in 1890. He concluded that the variations of urinary pepsinogen were so great that its estimation had no diagnostic significance but he waves this aside with no regret and with the hope that "Vielmehr mag es eine grosse physiologische Bedeutung haben". (Much more, it might have great physiological significance.).

It was with the failure of the German clinicians to apply this test to clinical medicine, and with the cautious interpretations of the more recent American authors in relation to ulcers in mind that I re-investigated the problem, guided by the words of Benderskey (1890) and planned the investigation from the view of possible physiological significance of disturbances of pepsinogen in gastro-duodenal ulceration, and in other diseases.

The main observations in this investigation were done on the disturbances of urinary pepsinogen excretion and only secondarily to that were the disturbances of blood pepsinogen investigated. These latter observations are far less complete and only capable of partial analysis. Most of this chapter will therefore deal with urinary pepsinogen excretion, expressed as rate of excretion in units per hour.

The first step was to establish normal levels of excretion of urinary pepsinogen and to determine the relation to sex and age. Having established that these factors do not influence excretion of pepsinogen in normal controls (see below) the disturbances of excretion could be analyzed in gastric and duodenal ulcers (Hirschowitz 1953). Other diseases were studied to a lesser extent, some only to elucidate physiological problems, e.g. renal disease.

Since it was obvious from previous papers and from early results that a simple analysis on conventional lines was not likely to yield any new information, it became necessary to subdivide material and results along slightly different lines. None of the previous authors had analyzed results by sex of the patient or by situation of the ulcer, though Janowitz, Levy and Hollander (1950) did separate their 5 gastric ulcers from the duodenal ulcers. They did not separate, as they should have, stomal ulcers from duodenal ulcers. Mirsky et al (1948) studied only males and did not specify the type of ulcer; nor did Varro et al (1952) but in any event their work can be discounted.

It was clear that ulcers excreted more urinary pepsinogen than controls but no hint of whether any factor could be responsible could be gleaned from these publications.

As will be seen later, the division of cases by sex and by site of the ulcer, and the later grouping of results by length of dyspeptic history held the clue to the real differences between ulcers and controls and may point the way to the Grosse physiologische Bedeutung" of disturbed urinary pepsinogen excretion predicted by Bemerskey in 1890.

RESULTS.

Urinary pepsinogen excretion was studied in 201 subjects and the figures givenare the mean of at least 2 separate sample of urine in each.

I. Control Group

This group comprised 60 persons, 36 males, 24 females, ranging in age from 15 to 76 years, and chosen from hospital staff and ambulant patients without gastric or duodenal ulcers or symptoms, to correspond approximately to the ulcer groups in age and sex distribution. The following tables show the information relevant to controls.

TABLE NO: 39a MALE CONTROLS.

URINARY PEPSINOGEN Hb (P.U.) /hr.	AGE IN YEARS 15-19	20-29	30-39	40-49	50-59	60-69	70 -	Total No.
0 - 99	-	1	1	1	4	2	1	10
100 - 199	1	1	2	3	3	1	-	11
200 - 299	-	3	1	2	1	-	1	8
300 - 399	1	-	-	1	-	2	1	5
400 - 499	-	-	1	-	-	-	-	1
500 -	-	-	1	-	-	-	-	1
Total No.	2	5	6	7	8	5	3	36

CONTROLS SUBDIVIDED BY SEX, AGE AND LEVEL OF URINARY PEPSINOGEN EXCRETION.

TABLE NO: 39 b FEMALE CONTROLS.

URINARY PEPSINOGEN (P.U.) Hb. /hr.	AGE IN YEARS							
	15-19	20-29	30-39	40-49	50-59	60-69	70 -	Total No.
0 - 99	-	-	1	1	1	-	1	4
100 - 199	-	1	-	3	1	2	-	7
200 - 299	-	-	2	2	1	-	-	5
300 - 399	-	-	2	1	1	1	-	5
400 - 499	-	-	-	-	-	1	-	1
500 -	1	-	-	1	-	-	-	2
Total No.	1	1	5	8	4	4	1	24

CONTROLS SUBDIVIDED BY AGE, SEX AND LEVEL OF URINARY PEPSINOGEN EXCRETION.

		AGE IN YEARS					
		15-29	30-39	40-49	50-59	60 -	Total
No. of Cases.	M.	7	6	7	8	8	36
	F.	2	5	8	4	5	24
Hb. Mean P.U./ /hr	M.	216	277	183	103	205	Mean. 195
	F.	360	213	243	267	236	244

TABLE NO: 40 CONTROLS: AGE DISTRIBUTION AND MEAN URINARY PEPSINOGEN EXCRETION.

The average excretion levels of the 36 males in 195 P.U./hr. S.D. ± 132 and the 24 females 244 P.U./hr S.D. ± 154. This difference is not significant at the 5% level ("t" = .514, V= 58, p >5%).

The age distribution was also compared statistically between the sexes and was shown to be comparable (t = .6, n= 2, p > .70). By inspection it can be seen that age has no consistent effect on urinary pepsinogen excretion. The only group that falls below the mean is the age group 50 -59 in male controls who excrete a mean urinary pepsinogen level of 103 P.U./hr., which is, however, within the range of the S.D.

Having shown that age and sex do not influence the excretion levels of pepsinogen in controls, the age distribution of the ulcer groups was compared to that in the controls. This showed that the groups were comparable by age and sex (Table No. 41)

TABLE No. 41. Age distribution in the various groups studied.

1. Males		AGE IN YEARS 15-19	20-29	30-39	40-49	50-59	60-69	70 -	Total no. of cases.	Mean age in years.
Control	a.	2	5	6	7	8	6	2	36	46.3
G.U.	b.	-	1	1	3	9	3	2	19	54.0
D.U.	c.	1	4	9	10	12	7	2	45	46.6
2.Females										
Control	d.	1	1	5	8	4	4	1	24	47.0
G.U.	e.	-	-	-	3	2	3	1	9	55.0
D.U.	f.	-	3	5	3	3	1	1	16	44.4

By inspection the age distribution will be seen to be similar and by statistical tests these are shown to be strictly comparable.

a cf. b	$\chi^2 = .102$	n = 1	$p > .70$
a cf. c	$\chi^2 = .460$	n = 2	$p > .70$
a cf. d	$\chi^2 = .600$	n = 2	$p > .70$
d cf. e	$\chi^2 = 2.25$	n = 1	$p > .20$
d cf. f	$\chi^2 = .164$	n = 1	$p > .50$

II. Gastric and Duodenal Ulcers.

This group comprises 61 patients with duodenal ulcers and 28 with gastric ulcers, with a mean excretion of 377 P.U./hr.

A. The duodenal ulcers will be dealt with first because they comprise a more homogeneous group with regard to the site of the ulcer. There were 45 males and 16 females in this group, ranging in age from 15 to 88 years. All were patients under the care of the department of Gastro-enterology, and all had had recent barium meals. All the ulcers were active in that the patients complained of pain or dyspepsia or had had recent bleeding or perforation of the ulcer treated by suture only. Presence of a coincident gastric ulcer or of renal disease excluded any patient from this group. For age distribution see table No. 41 .

The female excretion levels ranged from 58 to 594 P.U./hr and

75% of this group of 16 excreted less than 400 P.U./hr., and all less than 600 P.U./hr., the mean excretion level being 281 P.U./hr. S.D. ± 156. This was not different from the normal excretion levels for females.

The male excretion levels ranged from 56 to 1405 P.U./hr. and 62% of this group excreted under 400 P.U./hr., the mean being 435 P.U./hr. S.D. ± 379. This level was significantly higher than that in normal controls ("t" = 3.57, V = 79, $p < 0.1\%$).

B. Gastric ulcers. There were 19 males and 9 females in this category all of whom had active ulcers (as defined above) and all had been diagnosed by recent barium meal, gastroscopy or at operation. Presence of healed or active duodenal ulcers excluded any patient from this group. The excretion levels for the 9 females ranged from 94 to 660 P.U./hr. and all except one were below 450 P.U./hr. The mean excretion level for this group was 282 P.U./hr S.D. ± 211.8. This is not significantly different from that of female controls. The 19 male patients with gastric ulcers excreted between 41 and 912 P.U./hr., the mean excretion being 367.5 P.U./hr. S.D. ± 224. This level is nearer the duodenal ulcer level and significantly greater than for normal males. ("t" 3.2, $p < .01$).

POSSIBLE FACTORS INFLUENCING PEPSINOGEN EXCRETION IN PEPTIC ULCERS.

It was apparent even on simple inspection that the excretion levels for ulcers were different from those of controls but that the individual variations among the ulcers groups were very great and that many excretion levels were in the normal range. It then became pertinent to inquire into the possible factors within this group of ulcers that might reasonably divide these cases and allow of a satisfactory grouping.

The factors to be considered in evaluating this grouping were sex, age, activity of the ulcer, site of the ulcer, length of history and age at onset of symptoms.

It was found that of all these, only sex of the patient, site of the ulcer and length of dyspeptic history could be correlated with an altered level of pepsinogen excretion.

Let us first consider the factors that play no part in this disturbance.

(a) Age. As will be seen from Table 41 , the mean age of the duodenal ulcer groups was very close to that of the controls and that of the gastric ulcer groups a little higher (about 8 years). Nevertheless the groups were comparable(Table 41). In the group that shows the greatest individual variation within the group, the male duodenal ulcers, a partial correlation between age and pepsinogen excretion showed that if the length

of history were excluded as a factor, there was a negative correlation ($r_{us.h} = -0.0296$, $0.5 < p$). The same lack of correlation can therefore be assumed for the other sub-groups and the group as a whole. To confirm this, the following table shows an irregular distribution when age and excretion levels are compared.

	AGE IN YEARS							
	0-19	20-29	30-39	40-49	50-59	60-69	70 -	Totals.
0 - 99	-	1	2	2	5	2	2	14
100 - 199	1	2	2	6	4	3	-	18
200 - 299	-	3	3	4	2	-	1	13
300 - 399	1	-	2	2	1	4	-	10
400 - 499	-	-	1	-	-	1	-	2
500 - 749	1	-	1	1	-	-	-	3
750 - 999	-	-	-	-	-	-	-	-
1000 -	-	-	-	-	-	-	-	-
Totals	3	6	11	15	12	10	3	60

TABLE NO: 42 CONTROLS (BOTH SEXES)
AGE vs. URINARY PEPSINOGEN

	AGE IN YEARS							
	0-19	20-29	30-39	40-49	50-59	60-69	70 -	Totals.
0 - 99	-	1	-	4	3	3	2	13
100 - 199	-	1	4	5	8	1	1	20
200 - 299	1	1	3	2	3	1	1	12
300 - 599	-	3	2	2	3	4	-	14
400 - 499	-	-	2	1	3	2	-	8
500 - 749	-	1	1	3	2	2	1	10
750 - 999	-	-	3	-	3	-	-	6
1000 +	-	1	-	2	2	-	1	6
Totals	1	8	15	19	27	13	6	89

TABLE NO: 43 PEPTIC ULCERS (BOTH SEXES)
AGE vs. URINARY PEPSINOGEN

(b) Activity of the ulcer: It is conceivable that an active ulcer (as defined above) might cause more secretion of gastric secretions than an inactive ulcer. This was suggested by Professor C. Robb of St. Mary's Hospital, London, from his investigation into this subject (personal communication). However, none of this study was designed to enable one to determine this point. One can simply say that all the ulcers studied were active and despite activity much variation, shown to depend on other factors, was shown.

Activity, as defined above, included bleeding from the ulcer. Of 45 males with duodenal ulcers, 13 were admitted with haematemesis or melaena, 4 with histories under 5 years and the other 9 with histories longer than 5 years. The overall mean for the 13 cases was 481 P.U./hr; that for the 4 with histories under 5 years 231 P.U./hr., and for the other 9, 602.3 P.U./hr. The mean for the remaining 32 duodenal ulcers who had pain for which they were being treated at the time of the investigation (i.e. they all had some sign of ulcer activity) was 413 P.U./hr. These figures would indicate a small increase due to haemorrhage, or associated with the haemorrhage. The overall picture of a normal pepsinogen excretion with history of less than 5 years and an increase, irrespective of other factors except sex (see below) is still valid.

(c) Age of onset of symptoms. As with the age of the patient, this was found to have no constant relation to the increase in pepsinogen excretion in ulcers.

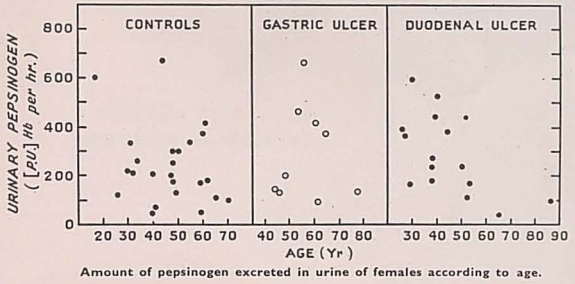
In considering the factors that do play a part in pepsinogen excretion in ulcers, sex of the patient, site of the ulcer and the length of dyspeptic history were all found to be important in the order mentioned.

(d) Sex of the patient. The usual practice in evaluating results in any clinical material is to examine the material for

any sex differences. This becomes even more important in dealing with peptic ulcers. It is well known that the incidence of duodenal ulcers is higher in males and that the course of the disease is often different between the two sexes. In gastric ulcers these differences are less well marked.

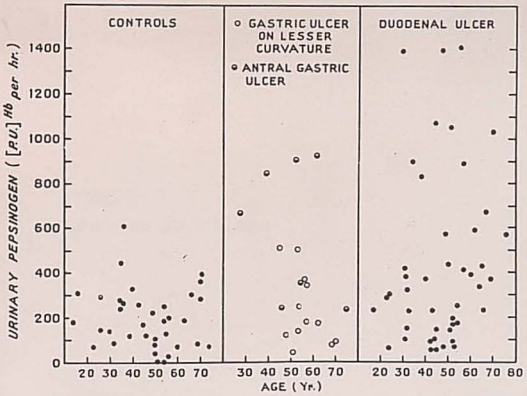
When pepsinogen excretion was analyzed by sex, striking differences were found. Of the 16 female duodenal ulcers all excreted less than 600 P.U./hr., and only 2 over 450 P.U/hr.; of the 45 males with duodenal ulcers, 13 excreted more than 450 P.U./hr. and 10 more than 600 P.U./hr. The mean levels for the 16 females was normal (281 P.U./hr) and for the 45 males significantly higher than normal (435 P.U./hr.) (see also fig 21).

Of the 9 females with gastric ulcers, only one excreted more than 450 P.U./hr.; of the 19 males with gastric ulcers, 6 excreted more than 450 P.U./hr.; the mean values for females and for males was 282 P.U./hr. as against 367.5 P.U./hr. (see also graph). The uniformly normal levels in females are not capable of further analysis and will be commented on later. Further observations, therefore, deal with male patients only.



Amount of pepsinogen excreted in urine of females according to age.

Fig. 20. Excretion in females. (Hirschowitz, 1953)



Amount of pepsinogen excreted in urine of males with different kinds of peptic ulcer and of controls.

Fig. 21. Excretion in males. (Hirschowitz, 1953)

(e) Site of the ulcer: This factor was used to analyze further the figures for excretion levels in male patients with ulcers. The only grouping of site possible was to differentiate duodenal from gastric ulcers and to separate gastric ulcers into those of the lesser curve and the pyloric ulcers. To separate the latter, radiological and gastroscopic evidence was assessed and the angulus was found to be a useful dividing point. In this way, of the 19 patients with gastric ulcers, 12 had ulcers of the lesser curve above the angulus and 7 had ulcers beyond the angulus in the pyloric portion of the stomach.

The 12 patients with lesser curve ulcers had a range of pepsinogen excretion from 41 to 515 P.U./hr., (mean 254 P.U./hr.) S.D.± 155) and were found by statistical test to be no different from male controls. ("t" .830, $0.05 < p < 0.10$).

The 7 patients with pyloric ulcers excreted considerably more than normal amounts of pepsinogen, the range being from 235 to 912 P.U./hr, mean 596 P.U./hr., S.D. ± 294 and this was found to be significantly higher than normal ("t" 5.06, $p < 0.001$).

The 45 males with duodenal ulcers could not be further subdivided by site. They excreted from 56 to 1405 P.U./hr., mean 435.5 P.U./hr., and this figure too was statistically higher than that of controls ("t" 3.57, $p < 0.001$).

Further analysis among these subgroups shows that the

lesser curve ulcers excrete significantly less than the pyloric or the duodenal ulcers, and that the pyloric ulcers are not significantly different from the duodenal ulcers (see table No. 44).

GROUP	NO. OF CASES	PEPSINOGEN P.U./hr.	S.D.
A. Controls	86	194	± 132
B. Lesser curve G.U.	12	234	± 155
C. Pyloric G.U.	7	597	± 295
D. Duodenal ulcers	45	435	± 379

By "t" test difference between	A & B "t"	0.830	$0.05 < p < 0.10$
	A & C "t"	5.06	$p < 0.001$
	A & D "t"	3.57	$p < 0.001$
	B & C "t"	1.06	$p < 0.01$
	B & D "t"	3.29	$0.02 < p < 0.05$
	C & D "t"	1.77	$0.05 < p < 0.10$

TABLE NO: 44 MEAN URINARY PEPSINOGEN EXCRETION IN MALES SUBDIVIDED BY SITE (Hirschowitz 1953) OF ULCER.

These results excluded lesser curve ulcers from further analysis since they were found to excrete normal levels. That left the 45 males with duodenal ulcers and the 7 males with prepyloric ulcers who resembled each other in excretion levels of

urinary pepsinogen, some of whom excreted normal amounts of pepsinogen and others a great deal more than normal.

From Table No. 44 it appears permissible to group pyloric gastric ulcers with duodenal ulcers as a functional group with similar excretion levels within the group.

(f) The length of dyspeptic history: This factor was the next that was applied to further analysis of pyloro-duodenal ulcers in males and as will be shown below, one which brings out the clearest differences between ulcer patients with a high and with a low urinary pepsinogen.

Length of dyspeptic history is taken as the continued presence of symptoms, i.e. pain or significant dyspepsia, or of complications of peptic ulcer or radiological or gastroscopic evidence of an ulcer without complete intermission of longer than 5 years (Hirschowitz 1953). As an example of this definition, a patient who had constant dyspepsia from 1927 culminating in a haematemesis in 1937, with a complete freedom of symptoms or complications until 1945, with a recurrence of pain and dyspepsia, would in 1951 have had a six year history only, because of the eight year interval from 1937 to 1945.

This analysis revealed that there was a definite and sustained increase in urinary pepsinogen in male patients with pyloro-duodenal ulcers with histories of longer than 5 years. For convenience, the patients were still kept divided into pyloric and duodenal. The following two tables plainly show

this increase related to length of history.

Urinary Excretion of pepsinogen P.U./hr.	DUODENAL ULCERS Dyspeptic history (yr.)							PREPYLORIC ULCERS. Dyspeptic history (yr.)	
	<2	2-4	5-9	10-14	15-19	20-24	25 or more.	<5	>5
1000 - 1500	-	-	-	2	1	1	2	-	-
750 - 999	-	-	2	-	-	1	-	-	3
500 - 749	-	-	-	-	1	2	1	-	1
400 - 499	-	1	-	1	2	-	-	-	-
300 - 399	3	1	1	-	1	1	-	-	1
200 - 299	2	1	1	1	-	1	-	2	-
100 - 199	3	5	-	-	-	-	-	-	-
0 - 99	5	1	-	-	-	-	1	-	-

TABLE NO: 45 PYLORODUODENAL ULCERS IN MALES ARRANGED ACCORDING TO LENGTH OF HISTORY (NUMBER OF CASES) (Hirschowitz 1953)

LENGTH OF HISTORY(yr.)	No. of cases.	Mean length of history (yr.)	Mean age (yr.)	Mean urinary excretion of pepsinogen P.U./hr.	
Duodenal ulcers					
0 - 4	22	1.34	45.2	186	a
5 - 9	4	6.7	34.0	594	b
10 - 19	9	13.9	47.7	694	c
20 or more	10	25.5	59.2	688	d
Total	45	10.52	46.6	435	e
Prepyloric gastric ulcer					
0 - 4	2	0.15	60.5	237	f
5 or more	5	7.8	46.2	741	g
Total	7	5.6	50.0	597	h

TABLE NO: 46 ANALYSIS OF RESULTS IN PYLORODUODENAL ULCERS ACCORDING TO LENGTH OF HISTORY (MALES) Hirschowitz 1953)

By "t" test: difference between a and b $p < 0.001$

a and c $p < 0.001$

a and d $p < 0.001$

h and e $0.02 < p < 0.10$

f and g $0.2 < p < 0.5$

It will be seen from Table No. 45 that the trend is from the lower left hand corner (short history with low urinary pepsinogen) to the upper right hand corner (long history with high urinary pepsinogen). This is further clarified and confirmed by Table No. 46 which puts this into actual figures showing that the patients with histories of under 5 years fall into the normal range (excretion levels are between 59 and 420 P.U./hr. in this group) and that the differences between this group and those withhistories of over 5 years are significantly different by "t" test at the 0.1% level.

Because of the suggestion in Table No. 46 that the ages of the patients in successive history groups was increasing, it became necessary to determine the effect of age in this particular group of patients.

The duodenal ulcer group of 45 was analyzed separately to keep to a homogeneous group and a partial correlation was employed to test this aspect.

This showed three things - firstly that age and length of history were closely linked. This correlation is obvious.

As had been anticipated the increase of pepsinogen, leaving age out of account, strongly correlated with length of history, and thirdly, that the correlation between age and level of pepsinogen excretion in the urine, leaving length of history out of account, is a negative one.

The figures are as follows:

Correlation coefficient

$$r_{uh.a} = 0.639 \quad , \quad p < .001$$

$$r_{ua.h} = -0.0296 \quad , \quad 0.5 < p$$

$$r_{ha.u} = 0.445 \quad , \quad p < .001 \text{ (Hirschowitz 1953).}$$

where u = urinary pepsinogen, a= age, h =length of history.

These figures prove beyond doubt that the increase observed in urinary pepsinogen excretion with an increasing length of history is a true one and is completely independent of the age of the patient. This is assumed to be true for the 9 pyloric ulcers as well.

This analysis by length of history was applied to the other groups and none of these was found to be related in any way.

	LENGTH OF HISTORY	
	Under 5 years	5 years or more
	P.U./hr.	P.U./hr.
Male duodenal ulcers	186	678
Male pyloric ulcers	237	741
Female duodenal ulcers	284	277
Female gastric ulcers	287	276
Male lesser curve G.U.	294	152

TABLE NO: 47 URINARY PEPSINOGEN RELATED TO LENGTH OF HISTORY (ALL GROUPS)

(g) The relation between gastric acid and length of history

This relation between length of history and urinary pepsinogen was found to be very interesting and felt to be of some importance. Accordingly fractional test meals of 60 males with gastric ulcers and 144 males with pyloroduodenal ulcers were analyzed from a similar point of view. This investigation into the gastric acid was on 204 male patients who were undergoing gastrectomy and in whom the ulcer was found at operation. The highest free hydrodloric acid of any of the specimens taken at 15 minute intervals in a gruel test meal lasting at least 2 hours was taken.(Hirschowitz and Kellock - unpublished observations).

TABLE 48: RELATION BETWEEN LENGTH OF HISTORY AND HIGHEST FREE ACID IN GASTRIC AND ANTRO-DUODENAL ULCERS,

(See text & fig. 22.)

Length of history (yrs.)	GASTRIC ULCER No. of cases.	HCl ml.N/10 % Lowest	Highest	Mean.	DUODENAL ULCER No. of cases.	HCL ml.N/10 % Lowest	Highest	Mean.
0 - 4	13	0	56	34.9	31	22	82	56.8 *
5 - 9	6	10	62	39.3	38	26	100	57.8
10 - 14	10	14	55	35	30	31	100	57.4
15 - 19	17	15	60	41.6	21	30	132	70.3
20 - 24	8	18	70	49.8	10	40	92	67.3
25 or more	6	22	60	40	14	51	92	75.3 *
Total or mean	60	13	60.5	41.1	144	33	99	61

* The difference between the duodenal ulcer groups 0 - 4 years and over 25 years is statistically significant ($\frac{D}{S.E.}$)

TABLE NO:

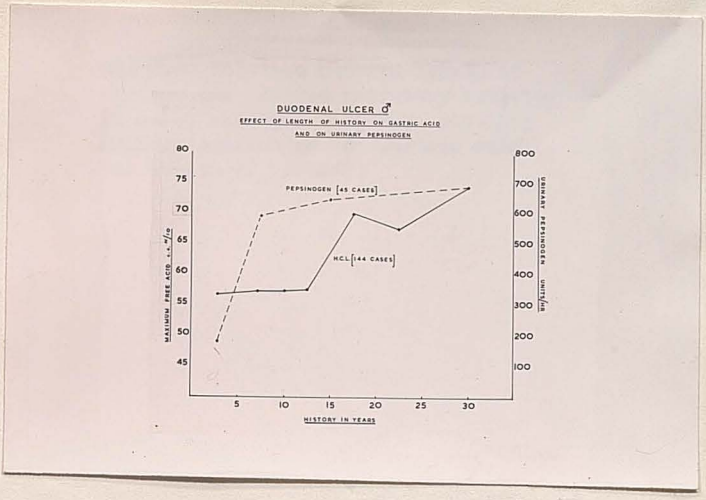


Fig. 22. Relation of gastric HCl and urinary pepsinogen to length of dyspeptic history in years.

These figures and the graph show that lesser curve gastric ulcers, whether measured by urinary pepsinogen or by gastric acid, excrete normal amounts of enzyme and secrete normal amounts of hydrochloric acid uninfluenced by the length of dyspeptic history.

Pyloroduodenal ulcers, on the other hand, show in both urinary pepsinogen and in gastric hydrochloric acid an increase with advancing duration of dyspeptic history. It is interesting to note that whereas the urinary pepsinogen is normal to start with, and rises to abnormal levels after 5 years, gastric hydrochloric acid is slightly above normal to start with but rises steeply after 15 years to abnormal

levels. The lowest free acid in both gastric and duodenal ulcers rises steadily.

This similarity between urinary pepsinogen excretion and gastric acid in relation to the length of dyspeptic history in males with duodenal ulcers is not only interesting but extremely important. It shows that gastric activity is probably normal at the time of the genesis of a duodenal ulcer and that the disturbances that have so commonly been associated with duodenal ulcers and which have been so very thoroughly investigated may merely be the result of the long-continued presence of the ulcer.

It has been shown in relation to urinary pepsinogen that sex and site of the ulcer are also fairly important. There were insufficient female patients for a reasonable analysis, but the similarity in finding normal gastric acid, unaffected by the length of history adequately confirms the findings of lesser curve gastric ulcer excretion of pepsinogen and further tends to confirm clinical differences between ulcers by site.

IN SUMMARY

Urinary pepsinogen estimations in 60 controls and 89 patients with peptic ulcers showed that ulcers as a group differed from controls in their levels of urinary pepsinogen excretion and that such differences, as were found were related to the sex of the patient (females excreted normal

amounts irrespective of other factors), site of the ulcer (lesser curve gastric ulcers excreted normal amounts of pepsinogen) and to the duration of dyspeptic history (patients with histories of less than 5 years excreted normal amounts.) Therefore the only patients who excreted abnormally high amounts of pepsinogen were those with pyloric or duodenal ulcers with dyspeptic histories of over 5 years.

PLASMA PEPSINOGEN

Experimental evidence relating to disturbances in plasma pepsinogen is given elsewhere in detail (p.130). It shows that in males the normal range is 0 - 45 P.U.%; gastric ulcers have a similar range and in duodenal ulcers the range is much greater - from 10 to 150 P.U.%; mean values also show this difference and for 15 controls is 18.46 P.U.%; for 11 gastric ulcers 27.6 P.U.% but in 27 duodenal ulcers 50.6 P.U.%.

The overlap between duodenal ulcers and controls and gastric ulcers is only 32% of duodenal ulcers; this suggests that plasma pepsinogen estimations might be of more value clinically than urinary pepsinogen.

III. URINARY PEPSINOGEN EXCRETION IN DISEASES OTHER THAN GASTRIC OR DUODENAL ULCERATION OR RENAL DISEASE.

This section deals with the results in 52 patients comprising 12 with pernicious anaemia, 8 with anaemias other than pernicious, 7 with stomal ulcers, 7 with carcinoma of the stomach, 12 with ulcer-like dyspepsias without demonstrable ulcers, 5 patients with exfoliative dermatitis, 2 patients with gastric atrophy and 1 with peptic oesophagitis and cardiospasm.

THE ANAEMIAS.

It has been repeatedly stated that in pernicious anaemia the urinary excretion of pepsinogen is zero or near zero, and these findings are in agreement with the absence of gastric juice hydrochloric acid and pepsin, though Helmer, Fouts and Zerfas (1954) state that in pernicious anaemia, gastric juice pepsin may not be entirely absent and may increase after adequate liver therapy.

The early authors, notably Bieling (1911) found pepsinogen in the urine in pernicious anaemia. One might discount these results, since the methods for diagnosis of pernicious anaemia were almost as crude as for determining urinary pepsinogen. Of the later workers, only Strauss (1928) found pepsinogen in the urine in such cases, despite the absence of gastric pepsin. He postulated the origin of this enzyme as somewhere other than the stomach and called it a "parasekretion". As I hope to show later, I agree with this view. Of later workers, Rothschild (1930) Farnsworth, Speer and Alt (1946) quoting 41 cases,

Podore et al (1948) quoting 9 cases, and Janowitz, Levy and Hollander (1950) quoting 7 cases, all found a verynlow or absent urinary pepsinogen. Farnsworth et al (1946) investigated the anaemias only and found that of their 41 pernicious anaemias, the concentration of urinary pepsinogen was 0 - 1.0 mg. (average 0.5 mgm) tyrosine equivalent per ml.; only 5 of 82 controls excreted less than 1.0 mgm. per ml., and of 14 true achlorhydrias, due to iron deficiency anaemia in females, 8 had values above 1.0 mgm. per ml. (range 0.3 to 3.2 mgm., average 1.2 mgm. tyrosine per ml.) The methods employed in that paper are not entirely accurate, though one cannot dispose of their results. They recommend the substitution of the gastric test meal by the estimation of urinary pepsinogen.

Of other macrocytic anaemias, only Janowitz et al (1950) investigated 2 patients with sprue and found normal levels.

RESULTS.

Of the 12 patients with pernicious anaemia studied, 9 were males and 3 females, ranging in age from 45 to 88 years. Pepsinogen excretion varied from 15 to 150 P.U./hr. (mean 82.5 P.U./hr.) and only 3 excreted more than 90 P.U./hr. Of the 8 with anaemias other than pernicious, 7 were females and 1 male. Excretion levels ranged from 105 to 370 P.U./hr. (mean 240 P.U./hr.). Only 2 excreted less than 150 P.U./hr., and none less than 105 P.U./hr.

PATIENT	TYPE OF ANAEMIA	CAUSE OF ANAEMIA	HIGHEST FREE GASTRIC HCl	URINARY PEPSINOGEN P.U./Hr.
B.C.	Macrocytic	Postgastrectomy steatorrhoea	0	105
J.L.(Male)	Megalo-blastic	Steatorrhoea	20	329
E.P.	Megalo-blastic	Not P.A.	0	248
S.S.	Megalo-blastic	Steatorrhoea	32	212
K.T.	Macrocytic	Myxoedema and steatorrhoea	0	184
I.H.	Microcytic	Iron deficiency	0	121
F.J.	Haemolytic	Marchiafava-Micheli	Not done	341
E.S.	Microcytic	Iron deficiency	Not done	370

TABLE NO: 49. Urinary Pepsinogen in non-pernicious anaemia.

It will be noted that there were 5 macrocytic anaemias, 3 of whom had megaloblastic marrows and 2 histamine fast achlorhydria. All responded well to folic acid but only partially or not at all to vitamin B.12. 4 of the 5 (excluding the post-gastrectomy anaemia) excreted 184 P.U./hr. or more.

From these figures it would appear that pernicious anaemia usually results in a low or nearly absent urinary pepsinogen which is not found in other macrocytic anaemias.

The single readings in some of the patients with pernicious anaemia, however, have not been uniformly low and a few isolated readings well in the normal range have been found in these 12 cases reported here. One patient in particular who had an undoubted pernicious anaemia that responded to Vitamin B.12, an absolute achlorhydria (both on histamine testing and on 24 hour pH test meal) and had a maximum of 760 P.U. % pepsin in hist gastric juice, gave the following results on 6 consecutive 24 hourly urines, starting on the 4th day of treatment. 27, 260, 422, 86, 45, and 34 P.U./hr. Whether this pepsinogen could have arisen from 1 pint of packed cells with which the patient was transfused on the first day is only a possibility. These results were all double-checked and gave identical results. In one other patient, 3 consecutive 24 hourly urines showed excretion levels of 345, 0, and 48 P.U./hr. This patient did not have a transfusion.

While these isolated normal readings are exceptional, they cannot be disregarded and would appear to invalidate the estimation of urinary pepsinogen as an absolute test for pernicious anaemia. The explanation of these high readings (for pernicious anaemia) might lie in the "parasekretion" theory of Strauss (1928) and perhaps the enzyme found arises from the white cells, which, as shown in Chapter III, form endogenous pepsinogen.

ANASTOMOTIC ULCERS.

7 patients with anastomotic ulcers were studied. All were admitted because of recurrent bleeding and operated on.

PATIENT	SEX	ORIGINAL LESION	PRESENT LESION	HISTORY (yrs.)	GASTROENTEROSTOMY (yrs.)	URINARY PEPSINOGEN P.U./Hrs.
C.O'B.	M	D.U.	S.U.	20	15	320
J.R.	M	D.U.	S.U.	15	10	292
R.S.	M	D.U.	S.U. and G.U.	11	10	305
H.B.	M	D.U.	S.U.	20	12	341
C.B.	F	D.U.	S.U. and G.U.	20	12	200
E.C.	F	D.U.	J.U. and D.U.	20	12	830
C.B.	F	D.U.	J.U.	40	12	243

D.U. Duodenal ulcer.
S.U. Stomal ulcer
G.U. Gastric ulcer
J.U. Jejunal ulcer

TABLE NO:50 URINARY PEPSINOGEN AND CASE DETAILS IN ANASTOMOTIC ULCERS.

These figures show that despite the long dyspeptic history, though many had been relieved of symptoms for a time, the urinary pepsinogen was not abnormally raised (except the one female E.C.) This would suggest that it is the actual presence of a duodenal ulcer for a long time which causes the rise in urinary pepsinogen excretion and that anastomotic ulcers should be grouped with gastric ulcers rather than with duodenal ulcers in their effect on the urinary pepsinogen.

CARCINOMA OF THE STOMACH

7 patients with carcinoma of the stomach were studied, 3 males and 4 females. The levels ranged from 45 to 294 P.U./hr., with a group average of 189. This shows that there is probably litle or no depression in the pepsinogen excretion as a result of gastric carcinoma. These results tend to confirm the fact that the gastric acid is not always depressed in carcinoma of the stomach. One other patient was studied, a 67 year old male. In May 1951 he had a gastric ulcer and an average excretion of 787 P.U./hr. In June 1951 he had deep X-ray therapy to destroy gastric secretion, which it did. In November 1951 he had no free acid and urinary pepsinogn excretion was 49 P.U./hr. Because of persisting pain, gastrectomy was performed - section showed a carcinoma.

X-RAY NEGATIVE DYSPEPSIAS.

In this group who were complaining of ulcer-like dyspepsia but in whom one or more barium meals had failed to reveal a peptic ulcer, there were 7 males and 3 females. The dyspeptic history ranged from 3 to 26 years.

PATIENT	URINARY PEPSINOGEN P.U./Hr.	HISTORY (yrs.)	SUGGESTIVE OF
1. H.G.	595	5	DU.
2. F.H.	568	5	D.U.
3 S.K.	454	23	H.H. and G.U.
4. R.P.	348	8	D.U.
5. D.Pe	387	10	D.U.
6. D.Po	268	= 3	P.U.
7. J.S.	283	= 5	P.U.
Average	386		

D.U. Duodenal ulcer
H.H. Hiatus Hernia
G.U. Gastric ulcer
P.U. Peptic ulcer

TABLE NO: 51a. URINARY PEPSINOGEN IN X-RAY NEGATIVE DYSPEPSIA (Males).

PATIENT	URINARY PEPSINOGEN P.U./Hr.	HISTORY (yrs.)	SUGGESTIVE OF
1. K.G.	252	12	D.U.
2. E.J.	59	23	? D.U.
3. G.L.	590	8	Hysteria
Average	300		

TABLE NO: 51b. URINARY PEPSINOGEN IN X-RAY NEGATIVE DYSPEPSIA (Females)

It is interesting to note that in this group there is some suggestion of an increased excretion of pepsinogen in the urine, although no ulcer could be demonstrated, either on barium meal or on gastroscopy. Several had melaena or haematemesis. It is quite likely that these patients actually had ulcers, and all were at one time or another clinically diagnosed as having some form of peptic ulcer. Most of them improved on an ulcer regime and one is therefore left to conclude that most so-called X-ray negative dyspepsias are, in fact, suffering from a peptic ulcer.

EXFOLIATIVE DERMATITIS.

This group provided some interesting results. There were 5 patients in this group, 4 of whom were of unknown aetiology and one the result of a generalized severe seborrhoeic dermatitis.

The 4 idiopathic exfoliative dermatitis cases were in a state of exacerbation of this disease; 2 were studied in remission as well as in relapse.

TABLE 52: URINARY PEPSINOGEN IN EXFOLIATIVE DERMATITIS.

PATIENT	URINARY PEPSINOGEN P.U./HR. IN RELAPSE	IN REMISSION.
W.H.	86	259
W.S.	24	198
J.F.	39	-
J.A.	121	-
W.D.(seborrhic dermatitis	116	-
Mean	77	

Patients W.H. and W.S. were treated with A.C.T.H.; W.H. in a quiescent phase in September 1951 excreted 259 P.U./hr. A.C.T.H. (1300 mgm. in 15 days) caused a drop from pre-treatment levels to 15 and 0 P.U./hr. on the 15th day and 17th day after the start of A.C.T.H. The dermatitis was little improved. Patient W.S. during his severest relapse in March 1951 excreted 24 P.U./hr. He was given A.C.T.H., 15 mgm. 6 hourly for 3 days with signs of rapid improvement and on the 4th day excreted 415 P.U./hr., on the 5th day 686 P.U./hr. and on the 7th day 345 P.U./hr. In October, 7 months later, he was almost normal and his pepsinogen excretion was then 198 P.U./hr.

The mean level in 5 patients with severe exfoliative dermatitis was found to be 77 P.U./hr. and in 2 in remission, 228 P.U./hr.

GASTRIC ATROPHY

One patient with gastric atrophy shown by histamine fast achlorhydria without any signs of pernicious anaemia but with a normal stomach on barium meal and gross mucosal atrophy on gastroscopy, excreted 14 P.U./hr. which is a negligible amount. The other patient with gastric atrophy caused by deep X-ray therapy excreted 49 P.U./hr.

These findings suggest that the stomach is the origin of most urinary pepsinogen.

CARDIOSPASM WITH PEPTIC OESOPHAGITIS

One patient (a female) studied, excreted a subnormal amount of pepsinogen (43 P.U./hr.)

THE DIAGNOSTIC SIGNIFICANCE OF PEPSINOGEN ESTIMATIONS.

Following the reports of Podore et al. (1948) and of Janowitz et al. (1950) it was hoped that the estimation of urinary pepsinogen might prove to be of some diagnostic value in gastro-duodenal ulceration.

The findings of normal levels in all patients except 60% of males with antro-duodenal ulcers of longer than 5 years standing, makes this test of only limited value. The following table, No.53 shows the excretion levels in gastric and duodenal ulcers and emphasises the limited diagnostic value of estimating pepsinogen in the urine.

(see Table No. 53 overleaf).

Urinary pepsinogen P.U./hr.	Control	Males			Females		
		L.C.G.U.	Antro-duodenal ulcers		Control	G.U.	D.U.
			5	5			
0 - 99	10	3	6	1	4	1	2
100 -199	11	4	8	-	7	4	4
200 -299	8	1	5	3	5	-	3
300 -399	5	2	4	4	5	1	3
400 -499	1	-	1	3	1	2	2
500 -749	1	2	-	5	2	1	2
750 -999	-	-	-	6	-	-	-
1000-1500	-	-	-	6	-	-	-
Total No. of patients	36	12	24	28	24	9	16

L.C. = Lesser curve

TABLE NO: 53. CASES SUBDIVIDED BY SEX, SITE AND LENGTH OF HISTORY AND ARRANGED ACCORDING TO EXCRETION LEVELS.

Plasma pepsinogen, however, reflects much more closely the actual activity of the stomach and also shows less overlap between duodenal ulcers and controls, suggesting that this test might be of some diagnostic value in duodenal ulcers, especially since the test is so easy to perform and far less uncomfortable than gastric analysis and less tedious than 24 hour urine collection. It might be of definite value in the patient who has been bleeding and in whom it is undesirable to do a barium meal. Blood urea estimations should be simultaneously performed to exclude high plasma pepsinogen due to renal disease. The following table shows the clear distinction between most of the duodenal ulcers and the controls.

TABLE NO: 54. PLASMA PEPSINOGEN LEVELS IN CONTROLS, GASTRIC AND DUODENAL ULCERATION AND IN PERNICIOUS ANAEMIA (NO. OF CASES).

P.U.%	Control	G.U.	D.U.	Pernicious anaemia
0 - 9	1	1	-	1
10 - 19	8	2	2	2
20 - 29	6	5	4	
30 - 39	3	3	4	
40 - 49	2	1		
50 - 59		1	3	
60 - 79			6	
80 - 99			6	
100 -150			2	
Total no. of cases	20	13	27	3
Mean P.U.%	23.2	26.3	60.4	16.3

With regard to the diagnosis of other diseases, it is felt that neither urinary nor plasma pepsinogen can be used with any measure of certainty.

The pepsinogen in the urine is occasionally useful in distinguishing a macrocytic or non-pernicious megaloblastic anaemia (especially if liver or vitamin B_{12} therapy has already been commenced) from a true pernicious anaemia.

Carcinoma of the stomach, as shown many years ago (Rothschild 1930) cannot be diagnosed by urinary pepsinogen.

It is of no value in X-ray negative dyspepsia and exfoliative dermatitis in relapse can be diagnosed without the aid of urinary pepsinogen estimations.

LIGHT THROWN ON THE AETIOLOGY OF PEPTIC ULCERS.

The universal use of antacids and of drugs, diets and operations designed to lower or eliminate gastric secretion in patients with peptic ulcers would seem to indicate a universal belief in the dictum that "acid and pepsin cause ulcers". The variety of treatments and the number of quacks merely underline the extremely poor results of treatment along these lines and emphasise the need to change the approach to the problem of peptic ulcers. The time has come to reconsider the value of the often misleading fractional test meal.

Very few people have seriously considered the possibility that peptic ulcers and hypersecretion may be only partly related, perhaps through a common "parent" or "grand-parent" stimulus.

The results obtained in this study lead one inevitably to believe that the latter possibility needs much closer attention.

Let us see what these results are and mean. If one takes urinary pepsinogen to indicate some aspect of gastric activity, plasma pepsinogen a similar one and gastric hydrochloric acid another, one is struck by the similarity between pepsinogen and hydrochloric acid in ulcers.

We find that both are normal in the following groups of patients:

(a) Females with gastric ulcers,

(b) Males with lesser curve gastric ulcers,

(c) Most females with duodenal ulcers,

(d) Some males with antro-duodenal ulcers,

and that both are definitely abnormal in most males with antroduodenal ulcers with histories of longer than 5 years.

In the males with antroduodenal ulcers of less than 5 years standing, none have abnormal pepsinogen excretion, though some have abnormal gastric acid and the mean gastric acid is at the upper limit of normal.

On that basis one may group peptic ulcers on a functional basis into

(a) lesser curve gastric ulcers,

(b) antroduodenal ulcers.

This fits in fairly well with the known clinical behaviour of these two groups of ulcers in all but the difference of incidence of carcinoma in pyloric (antral) and duodenal ulcers; though it is by no means certain that it is the antral ulcer which becomes malignant, rather than a coincidence of site.

The next subdivision on the basis of gastric function is that of sex - females secrete far less acid and pepsinogen (generally normal amounts) than males with antroduodenal ulcers. This corresponds to differences found clinically in incidence and behaviour between the sexes.

Thirdly the finding of increasing acid & urinary pepsinogen progressing with the length of dyspeptic history from 5 years onwards, but normal before that, leaves no doubt in my mind that when the ulcer starts, it does so with a normally secreting gastric mucosa.

These results suggest the following lines of thought:
(a) Does the difference between the secretory and clinical behaviour of gastric and duodenal ulcers signify that the ulcers are different or that the soil they are planted in by the unknown aetiological agent is different?
(b) What is the significance of the sex differences - does it imply that sex hormones play some part in gastric secretory activity, or merely that they modify the reactions of the stomach to the unknown aetiological agent causing the ulcer?

(c) Does the normally secreting stomach which is inflicted with a gastric or duodenal ulcer eventually hypersecrete (we have no evidence of any hypertrophy other than of Brunner's glands in duodenal ulcers) in response to the ulcer; or do both ulcer and hypersecretion follow the action of the unknown aetiological agent?; in either case, the lag of at least 5 years between ulcer and hypersecretion is a long time.

(d) One possibility which should be investigated is whether the rise of urinary pepsinogen and gastric acid with length of history may not be the result of alkaline treatment of the ulcer over the years. One would have to repeat the observations on plasma pepsinogen and investigate renal function in long-standing ulcers before being able to give a definite answer on these points.

(e) Is this unknown aetiological agent one thing or is it a combination of various psycho-endocrine factors and an abnormally reacting stomach or duodenum?

In concluding with these questions it is hoped that the need for a reorientation of thought towards the problem of the aetiology and behaviour of peptic ulcers will become more universal.

CHAPTER VI.

SUMMARY AND CONCLUSIONS.

This study was undertaken to investigate the significance of pepsinogen in health and in disease.

Pepsinogen, the inactive precursor of pepsin, is formed in the peptic cells of the stomach. It is mainly secreted into the stomach, where it is autocatalytically and irreversibly changed to pepsin and not reabsorbed. From the basal ends of the peptic cells a small amount of pepsinogen (about 1%) constantly diffuses into the blood-stream, where it may be found in the plasma (derived from the stomach) and in the leucocytes (formed endogenously).

Pepsinogen diffuses readily throughout the body and is freely filtered through the glomeruli and partly reabsorbed in the tubules. It appears in the urine at all ages and is relatively constant from day to day under varying dietary conditions.

The estimation of urinary and plasma pepsinogen offers a fairly reliable guide to the state of gastric secretion and it is found to be abnormally raised in the urine only in men with antro-duodenal ulcers with histories of more than 5 years duration.

The foregoing chapters present a new orientation to the physiology of pepsinogen and to the significance of its disturbance in disease of the gastro-intestinal tract.

THE PHYSICO-CHEMICAL PROPERTIES OF PEPSINOGEN.

Pepsinogen is a protein (mol. wt. 42,000 ± 3,000), which is enzymatically inactive but in acid medium it is rapidly autocatalytically and irreversibly converted to pepsin with the loss of about 15% of the nitrogen of the original molecule. Pepsinogen is reversibly denatured in very alkaline media and at temperatures of up to boiling point.

Pepsin and pepsinogen have some properties in common, but differ in those physico-chemical properties on which peptic activity appears to depend; they are also immunologically distinct.

METHODS OF ASSAY.

Pepsinogen can only be assayed after it has been converted to pepsin, and the techniques employed in peptic assay have been reviewed and criticised from both the theoretical and practical aspects.

The haemoglobin method of Anson and Mirsky (1932) was chosen for use in this study because of its reliability, its easy applicability to all peptic solutions and because it represents most nearly the true enzymatic action of pepsin. Several modifications of the method have been introduced to make the technique easy and practical for routine laboratory use. These modifications are all supported by experimental observations.

Briefly the method consists of the digestion of acidified haemoglobin substrate and the measurement of the increase in trichloroacetic acid-soluble tyrosine-like substances. One unit is the spectrophotometric equivalent of 1 x 10^{-4} mEq of l-tyrosine at 560mu.

An original method not previously described has been evolved to measure plasma pepsinogen and pepsinogen in similar fluids.

THE NORMAL PHYSIOLOGY OF PEPSINOGEN.

The secretion of gastric pepsin, the variations of pepsinogen in the blood and in the urine and the mechanism of pepsinogen excretion by the kidney were studied in relation to the normal physiology of pepsinogen.

a. Gastric pepsin.

Three different types of basal pepsin secretion were distinguished, the first showing a close relation between pepsin concentration and pH in hyposecretors, the second showing a close relation between the rate of secretion of pepsin and hydrochloric acid and the third showing no relation between the secretion of pepsin and the volume or pH of the gastric juice.

The stimulation of gastric secretion by either caffeine or histamine alone caused a small increase of pepsin, but the two together caused a marked sustained increase of pepsin secretion for some hours, indicating a true stimulation of pepsin, and not a simple washing-out of pepsin as has been previously assumed.

Correlation of simultaneous measurements of gastric pepsin and plasma and urinary pepsinogen revealed a fairly close direct relation between gastric and plasma pepsinogen, but not between gastric and urinary pepsinogen.

b. Plasma pepsinogen.

A proteolytic enzyme present in both the plasma and in the blood cells was identified as pepsinogen. Plasma pepsinogen derives from the peptic cells of the stomach by continuous diffusion, that of the leucocytes is probably partly formed endogenously.

The pepsinogen in the plasma is relatively constant under a variety of conditions and from day to day and the range in 20 normal controls was small (23.2 ± 10.8 P.U.%); 27 males with duodenal ulcers showed increased plasma levels (60.4 ± 31 P.U.%) and 13 gastric ulcers fell almost within the normal range (26.3 ± 10.6 P.U.%).

The estimation of plasma pepsinogen offers a simple and fairly reliable guide to the state of gastric secretion.

c. Distribution of pepsinogen in the body.

Pepsinogen has been detected in cerebrospinal fluid, in sweat, in pleural transudate and in pus. The concentration in the cerebrospinal fluid is about the same as that of plasma, indicating that pepsinogen is freely diffusable throughout the

body fluids, and suggesting that it has a molecular shape which lends itself to easy diffusion.

d. The renal mechanisms of pepsinogen excretion.

Results of this study show clearly that pepsinogen is not simply "freely excreted as are other waste products", but that it is filtered through the glomerular membrane and reabsorbed in the tubules to the extent of 60 - 98%. This is in accordance with the modern views on the renal excretion of other proteins. This concept of filtration-reabsorption of pepsinogen means that the evaluation of urinary pepsinogen is not complete unless one takes into account the role of the kidney as well as the state of gastric function, and variations in the individual of urinary pepsinogen can often be shown to be the result of changes in pepsinogen clearance by the kidney.

The significance of individual differences in urinary pepsinogen and exactly what aspect of gastric function it does measure, is by no means clear. That it does reflect, with relative constancy, some aspect of the function of the stomach, is certain, and one has merely to bear in mind its limitations to be able to take full advantage of its usefulness.

e. The effect of operations on pepsinogen.

Partial gastrectomy performed on 16 patients with gastric or with duodenal ulcers caused an enormous increase of urinary

pepsinogen for three to four days post-operatively - levels not seen in any other circumstance; this is partly accounted for by increased secretion of the fundal remnant of the stomach and partly by the diminished reabsorption in the kidney caused by altered haemodynamics.

Operations other than partial gastrectomy caused no postoperative increase of pepsinogen in the urine.

f. The adrenal and pepsinogen.

The effects of A.C.T.H. and of cortisone were studied in 10 cases and no consistent effect on the pepsinogen in the plasma or in the urine was noted, contrary to the previous reports.

Addison's and Simmond's disease also appeared to have no effect on urinary pepsinogen.

It appears therefore that the adrenals play little part in the regulation of gastric secretion.

DISTURBANCES OF PEPSINOGEN IN DISEASE.

(a) Peptic ulcers.

(i) Gastric and duodenal ulcers.

Urinary pepsinogen excretion was measured in 149 persons, 60 controls, 61 patients with duodenal and 28 with gastric ulcers.

All females, with or without an ulcer, excreted normal amounts of pepsinogen in the urine.

All male patients with lesser curve gastric ulcers excreted normal amounts of urinary pepsinogen.

The remaining male patients with ulcers beyond the gastric angulus and in the duodenum excreted, as a group, significantly more than normal amounts of pepsinogen in the urine. Within this group of antro-duodenal ulcers, all those with a dyspeptic history of less than 5 years excreted normal amounts of pepsinogen, whereas the rest excreted an abnormal amount which increased progressively with an increasing length of history to almost three times normal figures. This was found to be similar in the highest free HCl of 144 antro-duodenal male ulcers, but 60 gastric ulcer test meals resembled the pepsinogen figures for lesser curve ulcers in males.

(ii) Stomal ulcers.

7 patients in this group resembled gastric ulcers.

(iii) 10 patients with X-ray negative ulcer-like dyspepsia excreted slightly increased amounts of pepsinogen.

(b) Diseases other than gastric or duodenal ulcers.

(i) 12 patients with pernicious anaemia excreted about one third normal amount of urinary pepsinogen; whereas 8 patients with other anaemias (5 with non-pernicious macrocytic anaemia) excreted normal amounts.

(ii) 6 patients with carcinoma of the stomach excreted low normal amounts of pepsinogen.

(iii) 5 patients with exfoliative dermatitis excreted very small amounts of pepsinogen. 2 were studied in remission, when they excreted normal amounts.

It will be noted that only antro-duodenal ulcers with histories of over 5 years excreted increased amounts of pepsinogen in the urine and that this amount rises progressively with the length of history. The same was found true of the highest free HCl of 144 test meals in males with antro-duodenal ulcers.

It was originally hoped that the estimation of urinary pepsinogen might prove to be of some diagnostic value in gastro-duodenal ulcerations. Unfortunately, the finding of normal levels in all patients except 60% of males with antro-duodenal ulcers of longer than 5 years standing, makes this test of only limited value.

On the basis of findings in peptic ulcers it is suggested that further research might be useful into the sex differences, the effects of site of the ulcer and into the causes of the progressive increase in gastric acid and urinary pepsinogen with increasing length of history in antro-duodenal ulcers in males.

APPENDIX

MISCELLANEOUS OBSERVATIONS ON THE ACTION OF PEPSIN ON HAEMOGLOBIN

(a) The tyrosine content of haemoglobin

Complete digestion of 0.1 G. of haemoglobin by crystalline pepsin yields the maximum of 286 x 10^{-4} m.Eq. tyrosine and the mean for 7 observations is 275 x 10^{-4} m.Eq. 5 mg. tyrosine per 100 mg. haemoglobin.

This figure of 5% agrees well with the figures for tyrosine content of haemoglobin obtained by other methods.

(b) Solubility of tyrosine in the trichloracetic filtrate.

In digesting haemoglobin at pH ± 2 with strong concentrations of pepsin (1 mg. pepsin/0.1 G. Hb.) the filtrate after 5 minutes is opalescent and becomes progressively more opalescent up to a maximum after 60 minutes which appears to be crystalline tyrosine, which as we know is very insoluble, especially in strong acid solutions. (The pH of this filtrate is 1.40). This opalescence cannot be removed by repeated filtrations through filter paper and is not deposited by centrifuging at 4,000 r.p.m. The tyrosine is immediately dissolved in alkaline solution (pH 7.5). It is uncertain whether any of this crystalline tyrosine is carried down with the precipitate.

In any event, this is a further point in favour of not digesting haemoglobin with strong solutions of pepsin e.g. undiluted gastric juice.

(c) The production of haematin C.

When 0.1 G. haemoglobin is digested by 1 mg. pepsin as in (b)

above, a brown colour appears in the filtrate at 10 minutes and when digestion is continued at 37°C. this brown colour becomes progressively deeper in linear proportion to time as far as I have tested it (26 hours). This pigment flocculates on long standing in the cold and eventually settles to the bottom. It is insoluble in chloroform, ether or 20% HCl but precipitates at the separation layer in flakes.

Spectroscopic identification of the pigment showed a maximum absorption in the visual area of the spectrum at 560 mu, and some absorption in the ultraviolet range at 420 mu. The pigment obtained at over 20 hours contained a small amount of porphyrin. The pigment is not fluorescent.

A very similar if not identical pigment was made by mixing of acid haematin with cysteine. Tyrosine and tryptophane mixed with acid haematin did not reproduce the pigment.

In mixing cysteine and acid haematin, the colour of the latter changes from a reddish brown to the greenish brown colour of the pigment produced. In pyridine-sodium acetate solution this colour is even more pronounced.

This is one example of the production of haematin C., a term which denotes a number of loosely related compounds, studied in relation to cytochrome C., having in common the transformation of protohaematin into water-soluble, ether-insoluble compounds.

During peptic digestion of haemoglobin, the addition of amino-acids to the vinyl side chains (usually sulphur-containg amino-acids)

may occur, giving artifacts which on acid treatment yield a haematin C and porphyrin C. In this instance a special type of haematin C is produced where the combination is with cysteine. The linkage, then, is through the sulphur and not the nitrogen. This sulphur-containing compound is the only type of haematin C (orpporphyrin C) which closely resembles the corresponding products from cytochrome C (Lemberg and Legge 1949).

The addition of serum to the digest mixture (1 ml.) in four instances produced no change in the production of the haemtin C. but in one instance the serum from a patient with a gastric carcinoma after total gastrectomy prevented the formation of about 30% of the haematin C. This reaction is not understood.

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