

STUDIES ON THE GASTRIC JUICE PROTEIN

PART I POLAROGRAPHIC STUDIES ON THE PROTEIN OF THE GASTRIC JUICE IN PATIENTS WITH GASTRIC DISORDERS WITH SPECIAL EMPHASIS ON CANCER OF THE STOMACH

by

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INTRODUCTION

Little has been known about changes in gastric secretion, especially of the protein constituents, accompanying pathological conditions, such as mucosal deterioration in the case of cancer or precancerous states.

For a long time, authoritative opinion held that the protein, and even all the nitrogenous material of gastric juice, was represented exclusively by pepsin. The complexity of the protein component of gastric juice has only recently been recognized. During the past two decades several proteins of gastric juice have been described under various terms by different authors: Gastroglobulin (MARTIN¹⁾), second and third protein-like body (MARTIN¹⁾), mucoproteose (GLASS⁴⁾), mucoid and surface epithelium mucin (KOMAROV⁵⁾), mucoprotein (KOMAROV,^{2,3)} GLASS⁴⁾) and gastric peptide (SASAI⁶⁾) etc.

The whole issue has become very confused, due mainly to the lack of agreement in terminology, since in many instances, the same protein has been described under different names by different authors.

Evidence was first put forward by GLASS and his co-workers^{4,7-14)} that dissolved mucin is not itself a chemical entity, but consists partly of dissolved visible mucus, partly of a fraction called mucoproteose which is a mixture of degradation products of surface mucus, and partly of a fraction called glandular mucoprotein, which, it is suggested, is both a chemical and physiological entity, being derived from the mucus cells of the gastric glands. They not only found certain clinical significances in both mucoprotein and mucoproteose, but also made an interesting observation that the mucoprotein served as the intrinsic factor to combine with vitamin B₁₂^{9,15)}.

Studying the acetone supernatant fluid which had received little attention in the fractional precipitation method of GLASS and BOYD, SASAI^{16,17)} in 1956 found consistently detectable amounts of gastric peptide by the polarographic technique. Moreover, this peptide was found to be dialysable through cellophane membrane, and capable of inducing shock or anemia in rabbits when injected subcutaneously.

On the other hand IWATSURU¹⁸⁾ reported in 1937 that there was a "K.I.K. factor" (anemia causing principle) in the gastric juice of patients with cancer of the stomach. The entity of this substance has recently been found to be a peptide.^{19)~24)}

"Toxohormone" (liver catalase inhibiting factor), an extract obtained by NAKAHARA and FUKUOKA²⁵⁾ from cancer tissue, also proved to be a peptide.^{26)~33)} It was separated from body fluids of patients with cancer.³⁴⁾³⁵⁾

The polarographic protein wave technique described originally by BRDICKA³⁶⁾³⁷⁾ in 1933 found its clinical application in the polarographical cancer reaction of serum (Plague' reaction, BRDICKA,³⁸⁾ WALDSCHMIDT-LEITZ³⁹⁾ and others⁴⁰⁾⁴¹⁾). The entity of the polarographic-active substance in this sulfosalicylic acid filtrate of serum has recently been found to be mucoprotein by WALDSCHMIDT-LEITZ and MYER,³⁹⁾ WINZLER and co-workers.⁴²⁾ Polarographic examination of the serum protein was used in the study of the gastric juice protein by WADA et al.,^{43)~47)} SASAI⁶⁾ and KAKEI et al.¹⁶⁾¹⁷⁾⁴⁸⁾⁴⁹⁾⁵⁰⁾

GILLIGAN and others⁵¹⁾ also noticed the presence of many amino acids and peptide in the dialysates or in the ethanol filtrates of gastric juice.

The author employed the polarographic method using trivalent cobalt as the test solution, because of the advantage that the protein and peptide were the only polarographic-active substances present, regardless of the admixture of amino acid. In the present work efforts were made to clarify the origin and clinical significance of the gastric peptide and protein, with special emphasis on the nature and characteristics of these substances in cancer of the stomach with the hope of finding a method of early diagnosis of this malignancy.

I FUNDAMENTALS

This section describes the fundamental aspects of the entire work, i. e. material, methods and their critical evaluation, several expediences employed and their validity.

MATERIALS

Studies were carried out on subjects with gastric disorders other than gastric cancer, and those without gastric disorders.

METHODS

i) Aspiration of gastric juice

After fasting for about 12 hours, aspiration of gastric juice was made through a Rehfuess stomach tube before the ingestion of a caffeine test meal (Katch-Kalk method) and at intervals of 15 minutes thereafter for 2 hours. During aspiration of the specimens, the greatest possible care was taken to avoid contamination with saliva, blood or bile. In the following procedure, only juices not contaminated with blood, food or bile were used, except for 4 cases markedly contaminated with bile.

Juices were subsequently filtered through filter paper (Toyo Roshi, No. 5c) to remove visible mucus and other contaminants.

ii) Titration of acidity

The free and total acidity of each filtered juice was titrated by using as indi-

cator Topfer's reagent (0.5% alcoholic solution of dimethyl aminoazobenzene) and phenolphthalein (1% alcoholic solution).

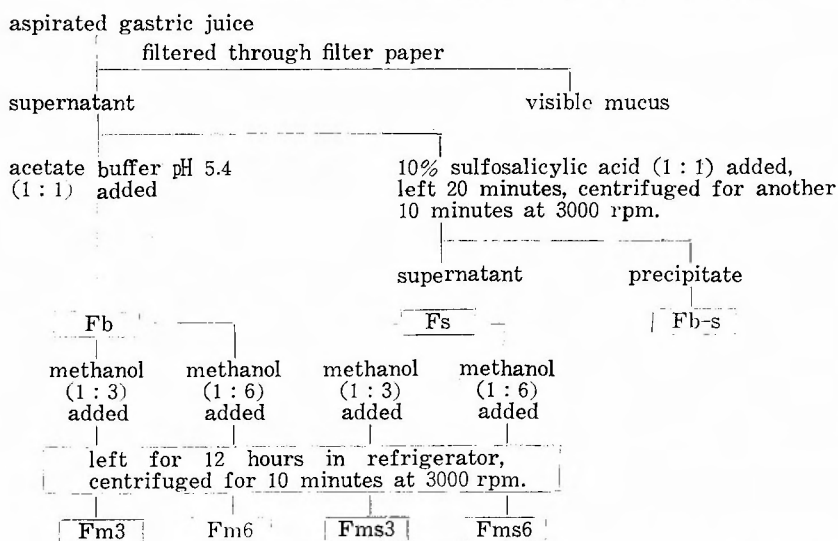
iii) Determination of protein content

The protein content of filtered juices was determined by MEHL'S⁵²⁾ modification of the Biuret reaction using sterile crystalline salt-free albumin as the reference standard, the values obtained being expressed in milligrams of serum albumin equivalent.

iv) Fractionation of gastric juice

Filtered juices were fractionated by the following method (Table I). 1.5 ml of each filtered juice sample was mixed with equal amounts of acetate buffer at

Table 1 Method of fractionation of gastric juice



pH 5.4. This buffered fraction was termed Fb. 2.0ml of Fb was divided into two test tubes in equal volumes. To one test tube was added three parts of 95% methanol. To the other tube was added six parts of 95% methanol. Both test tubes were closed with air-tight stoppers, vigorously shaken for a while, and kept in a refrigerator for about 12 hours. Then the precipitate was removed by centrifugation for 10 minutes at 3000 rpm. The two resultant filtrates were termed Fm3 and Fm6, according to the volume of methanol originally added. 1.5ml of each filtered juice was mixed with an equal amount of 10% sulfosalicylic acid, shaken for a while and kept for 20 minutes at room temperature and subsequently centrifuged for 15 minutes at 3000 rpm. This supernatant fluid and precipitate were termed as Fs and Fb-s, respectively. 2.0ml of Fs was divided into two test tubes in equal volumes and thereafter treated in the same way as fraction Fb, until reaching the final products, Fsm3 and Fsm6, which correspond to the previously described Fm3 and Fm6.

These six fractions were submitted to polarographic examination.

v) Taking polarograms

Six electrolysis cells were cleansed thoroughly and dried. Into each cell was

poured 5.0 ml of trivalent cobalt test solution which was made from the following standard stock solution prior to each examination.

(A) 1-N ammonium chloride

(B) 10^{-2} -M hexamminic cobaltic chloride (luteosalt) solution

(C) 1-N ammonium hydroxide solution

The test solution was prepared by adding in this order :

1 volume of A, 1 volume of B and 8 volumes of C.

To the first cell was added 0.1 ml of Fb and 0.5 ml of 95% methanol, to the second cell, 0.3 ml of Fm3 and 0.3 ml of methanol, to the third cell, 0.6 ml of Fm6, to the fourth cell, 0.1 ml. of Fs and 0.5 ml. of methanol, to the fifth cell, 0.3 ml of Fsm3 and 0.3 ml of methanol, to the sixth cell, 0.6 ml of Fsm6.

The dilution of the original gastric juice in each cell was nearly the same (1/112).

Polarograms were taken immediately in each cell as immersed in a constant temperature water bath at 18°C , with -0.8 volt to -2.0 volt. Sensibility of the galvanometer was 1/100. Drop time was 2 mg sec^{-1} . The wave height was measured in millimeters from the diffusion current of cobalt to the second maximum of the protein double wave. In the following paragraphs, the wave height of Fb, Fm, Fs and Fsm will be termed Fb value, Fm value, Fs value and Fsm value, respectively. The term Fm peptide and Fb-s protein will occasionally be used to indicate the proteid constituents in the methanol filtrate and protein precipitable by sulfosalicylic acid, respectively. The Fb-s value was calculated by subtracting the Fs value from the Fb value. For reasons stated elsewhere the terms Fm3 and Fsm3 have occasionally been simplified to Fm and Fsm, in this paper.

vi) Dialysis of gastric juice.

Polarograms were taken of normal acid gastric juice with and without dialysis which was carried out in a refrigerator for 24 hours. The dialysate was also studied. It was lyophilized and dissolved in distilled water to bring the volume up to the original (before dialysis), and studied polarographically after fractionations.

vii) Addition of blood or bile to gastric juice

0.2 ml, 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of 1.0% hemolyzed blood solution and 2.0 ml of A-bile aspirated through duodenal tube were added to 2.0 ml of acid and anacid gastric juice respectively, and incubated at 37°C for 15 minutes, and then polarograms were taken.

viii) Digestion of anacid gastric protein by HCl-pepsin. Anacid gastric juice was adjusted to pH 2.0 with 0.1-N HCl solution, crystal pepsin in a 10^{-3} concentration added incubated at 37°C for 15 minutes, and polarograms made.

The original anacid juice was also diluted with distilled water to the same volume and polarograms made.

RESULTS

i) Relation of Fb value to protein concentration

In Fig. 1, Fb values taken from 44 individuals are plotted against the respective

values of protein concentration. Fb values are almost linearly increased with protein concentration, except for 4 cases markedly contaminated with bile. The Fb values of these 4 exceptional cases are lower than those of the same protein concentration in uncontaminated gastric juice.

ii) Changes in Fb value related to ingestion of a caffeine test meal

The changes in both Fb value and free acidity value after ingestion of a caffeine test meal are shown in Fig. 2 which represents average curves drawn from 10 normal subjects. Both curves are similar except for the value of fasting juice. The average Fb values of fasting juice are higher than those after ingestion of a caffeine test meal, although both Fb values are intimately related.

iii) Comparison of the average wave height in six fractions

Among 142 examples the average wave heights of six fractions are shown in Fig. 3. It is rather unexpected that the Fsm value is much higher than the Fm value. This indicates that at least a portion of the protein constituents, presumably mucoprotein, mucoproteose or peptide in Fs, is soluble in methanol added to a strongly acid medium such as sulfosalicylic acid. In this case, even with the increased amount of methanol the Fm peptide can not be precipitable. In short, the Fsm3 value is equal to the Fsm6 value. The Fm3 values are usually somewhat higher than the Fm6 values.

iv) Comparison between acid and anacid gastric juices

Among 91 acid and 51 anacid examples the average wave heights of six fractions are shown in Fig. 4. A distinct difference is apparent from this figure in respect to Fs and Fm values. In anacid juices the Fm and Fs values are much lower than those in acid juices. In other words in anacid juices Fb-s protein is

Fig. 1 Relation of polarographic wave height to protein concentration of gastric juice.

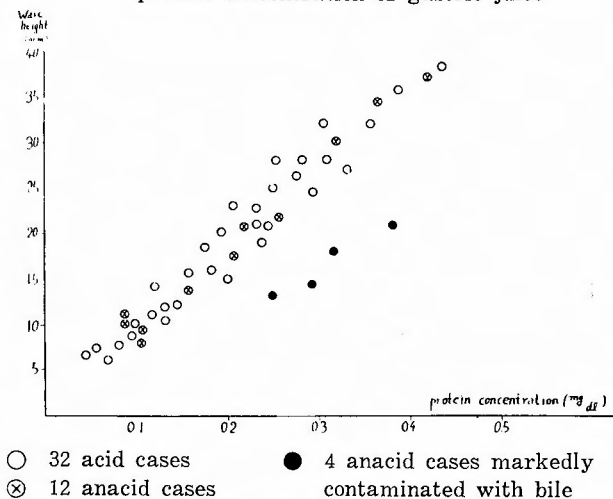


Fig. 2 Changes in Fb and free acidity values related to ingestion of caffeine test meal.

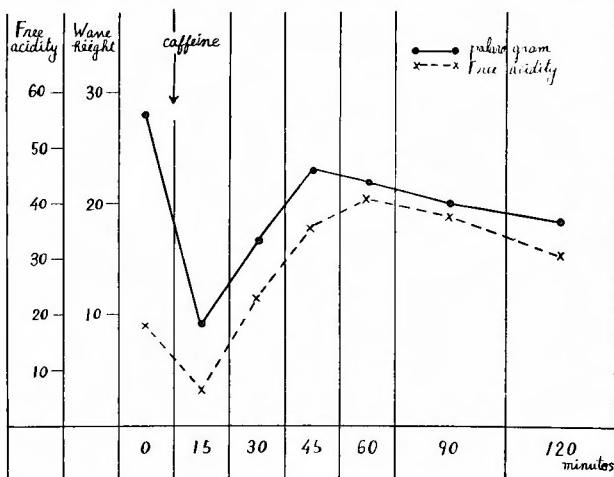


Fig. 3 Comparison of six fractions in average wave height (140 cases)

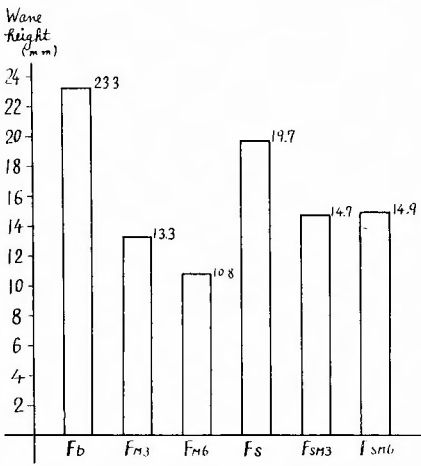


Fig. 4 Average wave heights of six fractions in acid and anacid juices.

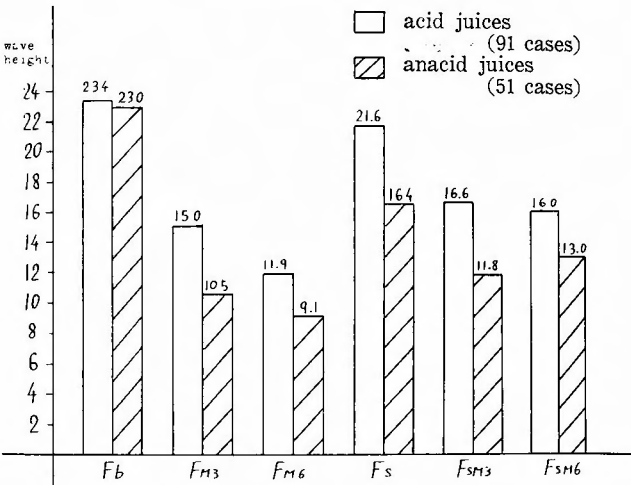
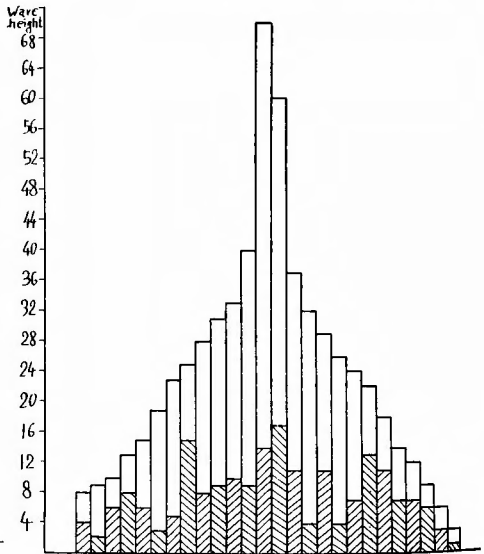
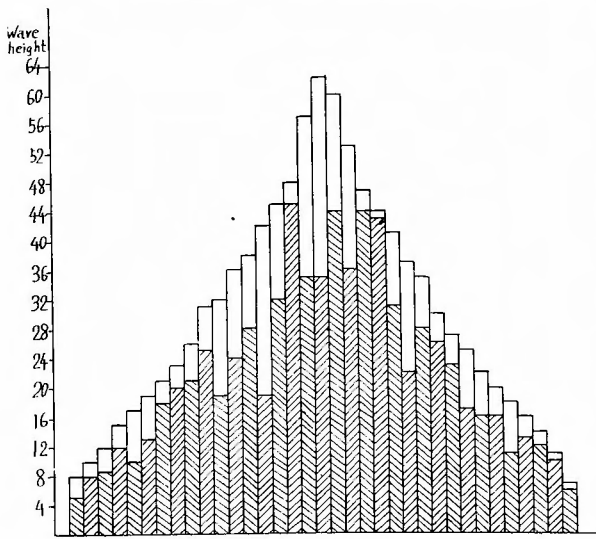


Fig. 5 Comparison between acid and anacid gastric juice with Fb and Fm value (shaded area : Fm value, shaded plus unshaded area : Fb value)
a) acid gastric juice (35 cases) b) anacid gastric juice (26 cases)



28.6% of the total high-molecular contents (Fb value) on the average. In acid juice, however, Fb-s protein averages only 7.7%. On the other hand Fm peptide in acid and anacid juices are 64.2% and 45.6%, respectively.

In Fig. 5 it is shown that in 35 acid specimens the Fm values constitute much more of the Fb values than in 26 anacid juice specimens. Each column represents one case.

v) Relation of Fb and Fm values to free acidity

In Fig. 6 Fb and Fm values from different subjects are plotted against the respective free acidity values. On the basis of data plotted in this figure, one might conclude that the Fb value is not related to free acidity, whereas the Fm value is not related to the amount of free acidity but to its presence or absence.

vi) Influence of dialysis on the wave height

Changes in the three values before and after dialysis are shown in Table 2. The Fm value before and after dialysis changes from 20mm to 0mm. Three values of the dialysate are nearly 16 mm. This indicates that Fm peptide is dialysable.

vii) The influence of contamination with blood or bile on the wave height

In Table 3 it is shown that both acid and anacid Fb values are increased in proportion to the amount of added blood except when the concentration of blood is less than 0.25%.

Polarograms taken from normo-acid and anacid juice, and taken from juice contaminated with blood at 1.0% concentration or bile at 50% concentration are

Fig. 6 Relation of Fb and Fm value to free acidity (56 cases).

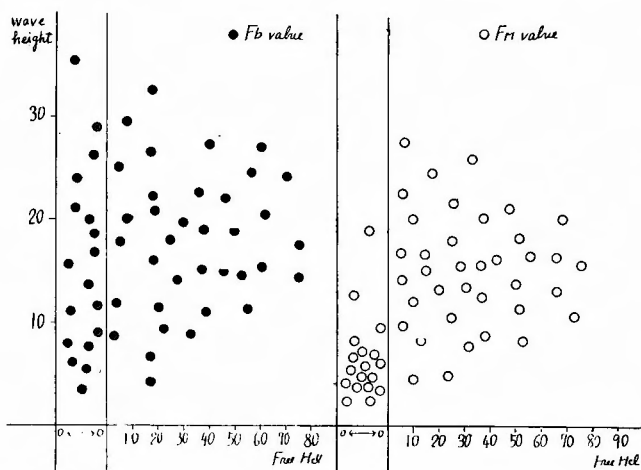


Fig. 7 Influence of contamination with blood and bile on wave height and form.

Table 2 Change in the 3 values before and after dialysis.

	before dialysis	after dialysis	dialysate
Fb value	29.0	6.0	16.0
Fm value	20.0	0	15.7
Fs value	27.4	5.8	15.9

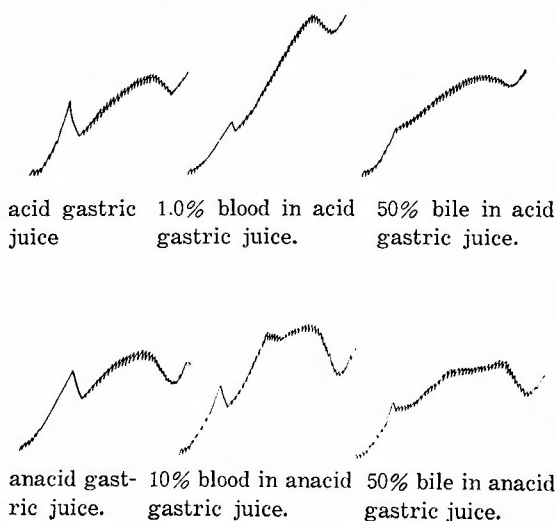


Table 3 Relation of amount of added blood to Fb value of acid and anacid gastric juice.

Blood concent. in juice (%)	Fb Value of acid juice (mm)	Fb Value of anacid juice (mm)
0	17	15
0.1	17	15
0.25	17	16
0.5	21	18
0.75	29	20
1.0	40	25

shown in Fig. 7. It is interesting that polarograms made from gastric juice, blood and bile show different wave forms. Polarograms made from bile show a peculiar form with a very low cobalt maximum.

viii) Influence of HCl-pepsin digestion of anacid juice on wave height and form

Changes of wave height and form before and after HCl-pepsin digestion of anacid juice are shown in Fig. 8. The wave form of Fb is changed into a different wave form by digestion. Fm and Fs values after digestion are strikingly increased. This indicates that Fm peptide is increased as a result of the addition of HCl-pepsin to anacid juice proportionately to the decrease of Fb-s protein.

DISCUSSION

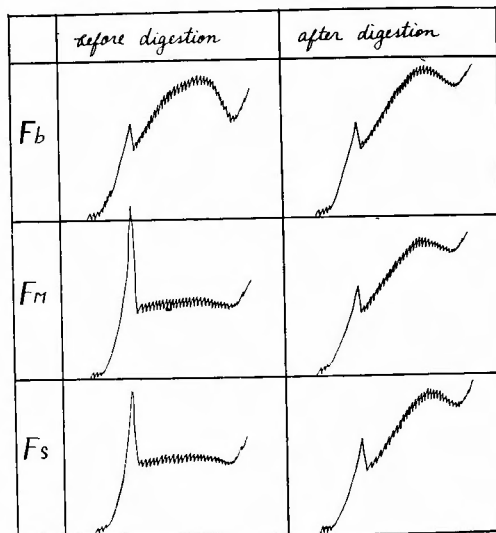
It has already been reported by NECKI and SIEBER,⁵³⁾ and lately by MARTIN¹⁾ that various protein constituents are present even in the dialysate of gastric juice. Indeed, in the methanol filtrate (Fm) of gastric juice a dialysable protein is found polarographically. Because of its physico-chemical properties this proteid was called gastric peptide by SASAI.⁶⁾¹⁶⁾¹⁷⁾ In this paper it is represented as Fm peptide. This Fm peptide is more abundant in acid gastric juice than in anacid juices, and represents a considerable part of the total high-molecular constituents in acid juices.

Fb values represent the total amount of high-molecular constituents of mucoprotein,⁴⁾ mucoproteose,⁴⁾ Fm peptide and protein precipitable by sulfosalicylic acid (Fb-s protein) in gastric juice. Because the methanol precipitate is related to the pH of gastric juice, acetate buffer is added to filtered juice. Moreover, Fm is preferable to Fsm as fractions of the methanol filtrate, because at least a portion of the protein constituents, presumably mucoprotein, mucoproteose or peptide in F's, is soluble in methanol added to a strongly acid medium such as sulfosalicylic acid.

On the other hand Fb-s protein is found only in anacid specimens. Since an increased amount of Fm peptide is found after addition of HCl-pepsin to blood or anacid gastric juice, it seems likely that Fm peptide is closely related to the breakdown-products of Fb-s protein formed by the enzymatic action of pepsin.

Fb-s protein is probably the same protein as was separated from human gastric juice by precipitation with 1/2 volume of 10% trichloroacetic acid and named by GLASS⁴⁾ dissolved visible mucus. There is evidence that mucus undergoes chemical degradation in the stomach, losing its viscosity in the process. How this degradation is brought about is still uncertain; pepsin itself is known to digest mucus, but it is

Fig. 8 Change in wave height before and after HCl-pepsin digestion of anacid gastric juice.



clear from the studies of GLASS and BOYD⁵⁴⁾ and of JANOWITZ and HOLLANDER⁵⁵⁾ that mucus becomes liquefied at or near neutrality, when pepsin would be inactive. As it is liquefied it becomes converted into a fraction soluble in 10% trichloroacetic acid which GLASS and BOYD⁴⁾ call mucoprotease. But it is also evident that there is a large amount of Fb-s protein in anacid gastric juice in addition to mucoprotease. It seems more likely that Fb-s protein in anacid juice is not only liquefied visible mucus, but also is mucus secreted in a water soluble form by columnar surface epithelial cells or cells of pyloric and cardiac glands.

Fm peptide and Fb-s protein are completely neglected by the fractional precipitation method of GLASS⁴⁾ which is receiving increasing attention by many investigators.

It would seem that the difference between Fs and Fm values corresponds to mucoprotein and mucoprotease.

But mucoprotein and mucoprotease cannot be determined accurately by this polarographical fractional method.

The wave heights of fasting juices are higher than those after ingestion of a caffeine test meal. One of the reasons for this difference may be that gastric juice is diluted by ingestion of a caffeine test meal. These data agree with KANAZAWA's data.⁴⁴⁾

The influence of contamination with blood on the wave height of the polarogram is very important especially in clinical analysis, because gastric juices aspirated from patients with gastric cancer are frequently contaminated with blood. When blood is added to acid gastric juice, the Fb, Fm, and Fs values all increase markedly, but blood in anacid juice increases the Fb value only. It is evident that in acid juice blood is digested by pepsin and broken down to peptides. When contamination is not macroscopic, it has no influence on the wave heights, even when the gastric juice shows a positive benzidine reaction. These data agree with UMETANI's data.⁴⁶⁾

Because of the frequent contamination of fasting juice with blood, bile or food, the sample aspirated 45 minutes after ingestion of a caffeine test meal is preferable for clinical investigation.

The polarographic activity of bile is lower than that of gastric juice. Although the polarographic activity of protein originates in the thiol or disulfide group, this observation will not warrant an off-hand inference as to the thiol activity involved, because other factors, for instance the protein aggregate volume, can modify the situation.

Moreover, it is very interesting that there is a difference between gastric juice and bile or blood with respect to the wave shape of the polarogram. BALL-HELAERS⁵⁷⁾ and SASAI⁵⁸⁾ reported that the first maximum of a protein double wave originates in general in the protein-bound polysaccharide. The characteristics of the wave form may be studied as an important approach to the problem of the structure of the protein molecule.

On the polarogram of bile the cobalt maximum is very low or not present at all. This indicates that there are substances inhibitory to the cobalt maximum in bile. One of these substances is presumably bile pigment, because inhibition of

cobalt maximum is closely related to surface active agents.

II CLINICAL OBSERVATION

On the basis of the experimental results described above clinical observations were carried out.

MATERIALS

Studies were made on 283 subjects hospitalized in the 2nd Surgical Clinic of the Medical Faculty of Kyoto University, with the following diseases: gastric cancer 114, peptic ulcer 43, duodenal ulcer 33, gastric ptosis 14, chronic gastritis 5, gastric polyp 4, gastric leiomyoma 1, post-gastrectomy 4, other gastric diseases 35, various illness other than gastric disturbances 30. Of the 253 cases of gastric disorders the diagnosis was confirmed in 200 cases by surgical operation. The resected stomach tissues in addition to tumor if present were examined by histological and at times by histochemical techniques. The relation between histological finding of gastric mucosa or tumor itself and protein in gastric juice will be reported elsewhere by SHINOHARA.

METHODS

i) Aspiration of gastric juice was 45 minutes after ingestion of a caffeine test meal as described above. Contamination with blood, bile and saliva was minimized by strict precautions taken during aspiration of the juice, and checked if present. The aspirated juice was filtered by filter paper (Toyo Roshi, No. 5c) to remove visible mucus, and the benzidine test for occult blood in the juice was done.

ii) Titration of acidity.

The free and total acidity values of filtered juice were measured as described above, and classified as hyper-, normo-, hypo-, and anacid if the free acidity values were 39 or more, 20-39, 1-19, and 0, respectively.

iii) Filtered juice was fractionated into 3 (Fb, Fm, Fs) fractions as described above.

iv) Polarograms were made of the 3 fractions as described above.

v) Classification of polarograms.

Polarograms were classified into the following three types with regard to Fb and Fm values.

Type I:

Fb, Fm and Fs values all were over 25 mm. In other words Type I represented cases where the high protein values were ascribed to increased amounts of gastric peptide.

Type II:

In spite of low values of less than 25mm of Fm peptide, Fb values were over 20mm. In other words Type II represented the cases where high protein values were due to an increased amount of protein precipitable by sulfosalicylic acid.

Type III:

Fb, Fm and Fs values all were less than 20mm. This type indicated that all protein components in the gastric juice were very low.

RESULTS

i) Comparison between cancer cases and non-cancer cases in respect to the benzidine reaction of gastric juice. Cancer and non-cancer cases are compared in terms of the incidence of positive benzidine reactions as shown in Table 4. In cancer cases benzidine reactions are at least 3 times as often positive as in the non-cancer group.

Table 4 Benzidine reaction in cancer and non-cancer group.

	Total cases	cases of positive Benzidine reaction	Rate (%)
Cancer group	114	68	59.6
Non-cancer group	169	33	19.6

ii) Relations between acidity and gastric diseases are shown in Table 5. In cancer cases anacid and hypoacid cases are 54.4% and 30.7%, respectively, while in non-cancer cases anacid and hypoacid cases are 19.5% and 20.6%, respectively. With duodenal ulcer hyperacid cases are 63.7%. All cases of gastric polyp are anacid.

Table 5 Relation between acidity and gastric diseases.

	anacid cases	hypoacid cases	normoacid cases	hyperacid cases	Total
gastric cancer	62	35	14	3	114
peptic ulcer	3	13	10	17	43
duodenal ulcer	0	6	6	21	33
gastric ptosis	2	4	2	6	14
chronic gastritis	4	0	1	0	5
gastric polyp (Leiomyoma)	5 (1)	0	0	0	5
after gastric resection	2	0	0	2	4
other gastric disease	7	9	9	10	35
others	10	3	12	5	30
Total	95	70	54	129	283

Table 6 Relation between polarogram types and acidity.

	Type I		Type II		Type III	
	acid	anacid	acid	anacid	acid	anacid
cancer group	34	8	10	35	7	19
non-cancer group	29	2	51	13	56	18

iii) Relation between 3 types of polarograms and the presence of acidity in cancer and non-cancer cases are shown in Table 6. It is evident from this table that in cancer cases the presence of free acidity is closely related to types of polarograms. On the other hand in non-cancer cases there is no such relation.

iv) Comparison between cancer cases and non-cancer cases with regard to 3 values of polarograms. 3 average values each of cancer and non-cancer cases are shown in Table 7. A significant difference is found between cancer and non-cancer

Table 7 3 values of polarograms in cancer and non-cancer groups.

	Type I			Type II			Type III		
	Fb Value	Fm Value	Fs Value	Fb Value	Fm Value	Fs Value	Fb Value	Fm Value	Fs Value
gestric cancer	43.3	39.3	39.7	31.0	13.7	17.7	12.6	8.4	9.1
	42 cases			45 cases			26 cases		
non-cancer	33.5	28.6	31.3	25.2	15.8	21.1	13.9	8.9	11.6
	31 cases			64 cases			74 cases		
peptic ulcer	34.2	28.6	33.5	25.0	18.4	23.1	14.6	10.8	13.6
	18 cases			14 cases			11 cases		
duodenal ulcer	32.2	26.7	29.2	25.5	15.2	23.3	14.9	10.5	14.7
	5 cases			10 cases			18 cases		
gastric ptosis	25.0	25.0	25.0	27.8	20.4	24.2	13.0	8.6	11.2
	1 cases			5 cases			8 cases		
chronic gastritis				26.3	10.3	12.3	10.5	5.5	8.0
	0			3 cases			2 cases		
benign tumor (polyp, myom)				26.0	9.3	16.3	16.5	5.0	6.0
	0			3 cases			2 cases		
other gastric diseases	29.8	30.8	31.2	27.1	17.2	24.7	13.4	8.8	11.6
	5 cases			16 cases			14 cases		
others	29.0	36.0	25.5	24.7	13.6	16.6	12.4	8.5	10.6
	2 cases			11 cases			17 cases		

cases with regard to Type I as well as Type II polarograms. With regard to Type I, cancer cases give higher average values for Fb, Fm and Fs than do the non-cancer, the differences being 9.8 mm, 10.7 mm and 8.4 mm, respectively. With regard to Type II, cancer cases give higher Fb values and Fb-s values than do non-cancer cases. There is no difference in Fm values. With regard to Type III, there is no difference between cancer cases and non-cancer cases as to Fb, Fm and Fs vlues. Cancer cases of this type are 27.8% of the total cases.

v) In Fig. 9 cancer cases of Type I are compared with acid non-cancer cases (peptic ulcer, duodenal ulcer, gastric ptosis and others) in terms of Fm value. In this scattergram cases of early stage of gastric cancer and precancerous states and ulcus callosum are plotted with a different mark (▲).

vi) In Fig. 10 cancer cases of Type II are compared with anacid non-cancer cases (gastritis chronica, gastric polyp and others) in terms of Fb value.

DISCUSSION

Since GOLDING BIRD (1842) first described decreasing acidity in the vomitus of a gastric cancer patient and VON DEN VELDEN reported the frequent association of

Fig. 9 Comparison between cancer cases of Type I and acid non-cancer cases with regard to Fm value.

▲ cases of early stage of gastric cancer precancerous state and ulcer callosum

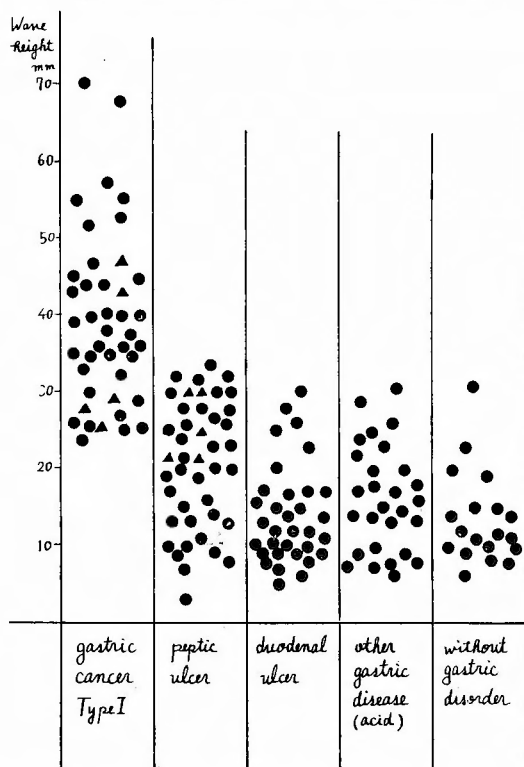
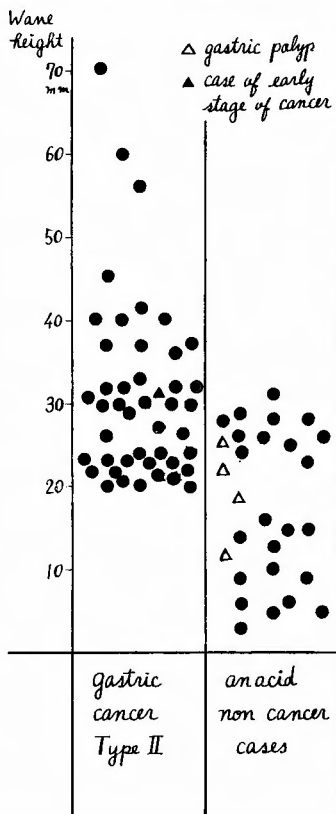


Fig. 10 Comparison between cancer cases of Type II and anacid non-cancer cases with regard to Fb value.



achlorhydria with gastric cancer (1879), a huge literature has accumulated not only of achlorhydria associated with gastric cancer but of achlorhydria itself. During the past 30 years the reported frequency of achlorhydria in gastric cancer has varied from 50 to 69%.⁵⁹⁾⁶⁰⁾⁶¹⁾ Gastric cancer has been stated to occur 3 to 5 times more frequently in the achlorhydria individuals of comparable age groups, and also to occur 10 times more frequently in the achlorhydria individuals over 40. In the author's data frequency of achlorhydria in gastric cancer is 54.4% and is 2.8 times that of non-cancer cases. Moreover, all cases of gastric polyp which are precursors of gastric cancer occur in patients with achlorhydria. These statistics and the author's data have figured prominently in the rationale of examination of gastric acidity in the diagnosis of gastric cancer.

There is another difference between cancer and non-cancer cases in respect to the benzidine reaction of gastric juice. In cancer cases the frequency of positive benzidine reaction is about 60%, in non-cancer cases it is only 20%. Because contamination with blood affects the wave height of polarograms as described above, cases markedly contaminated with blood are not included.

Significant differences are found between cancer and noncancer cases with regard

to wave height of polarograms. All cancer cases of Type I and II show higher protein average values than do non-cancer cases either in Fb or in Fm values, depending on whether the gastric juice specimens are acid or anacid.

With regard to Type I in cancer cases, gastric peptide increases more than in non-cancer cases. Moreover, cases in the early stage of cancer, precancerous states and ulcus callosum give high peptide values. Peptide values of over 35mm occur exclusively in cases of gastric cancer.

Some cases with peptic ulcer sometimes give high peptide values. About the relationship of peptic ulcer and gastric cancer a great deal has been said and written. At this point the author's data offer some additional information.

With regard to Type II, cancer cases give higher values of Fb and lower values of Fs than do non-cancer cases, and are closely related to achlorhydria, and Fb-s values also increase. Fb values over 30mm occur only in cancer cases. However it must be stressed that the clinical course of Type I cancers is no less malignant than that of Type II cancer, despite the presence of free hydrochloric acid and the relatively young age of the patients in the former case.

Type III cancers seem to be in the most advanced and so inoperable stages. Cases with severe atrophic gastritis give the lowest protein values. Therefore it seems probable that in Type III cancer cases damage or atrophy of the gastric mucosa is more severe than in cancer cases of other types.

The protein wave due to methanol filtrate (Fm), which may correspond to protein in dialysates and also to fraction V of WADA and coworkers,⁴⁷⁾ is apparently caused by peptide mixture, although not homologous. GILLIGAN and others⁵¹⁾ have already noticed the presence of peptide in dialysates or in ethanol filtrates of gastric juice specimens, utilizing paper chromatography. But they did not refer to its clinical significance. OHUCHI and AWATAGUCHI⁶²⁾ applying resin-chromatography to gastric juice have also found a peptide in cases of gastric cancer, but the data are still too scanty to discuss.

It is amply demonstrated in this paper that the polarographic protein wave as tested with trivalent cobalt solution is most suitable for the estimation of peptide, because only peptide exhibits polarographic activity regardless of the admixture of amino acids. This peptide is pepsin digestive products of protein precipitable by sulfosalicylic acid as above described. It seems more likely that in gastric juice from patients with gastric cancer, the amount of protein, (which is not secreted specifically by the cancer itself, but by non-specific altered mucosa of the secondary gastritis accompanying gastric cancer), increases so that the amount of peptide increases in acid juice and the amount of protein precipitable by sulfosalicylic acid increases in anacid juice, although a high protein value in cancer cases is partly related to an absence of the ordinary diluting effect of watery acid glandular secretion. With regard to the denaturation of gastric protein WADA and coworkers^{46/47)} have reported that the gastric protein of anacid cancer specimens is usually labile and its polarographic protein wave is markedly increased when hydrochloric acid is added. These data probably indicate that protein precipitable by sulfosalicylic acid,

presumably soluble mucus according to GLASS,⁹⁽¹⁰⁾ is easily denatured or degraded by hydrochloric acid and pepsin. With regard to soluble mucus, GLASS⁹⁽¹⁰⁾ reported that soluble mucus can be precipitated with trichloroacetic acid. A substance of similar physical properties is obtained on physical dissolution of surface epithelium mucus or from its initial liquefaction on incubation. Therefore it represents most probably a liquefied fraction of the visible mucus which is physically dissolved in the gastric juice before it undergoes further degradation. The possibility cannot be excluded however that some part of it represents preformed fluid mucus secretion, which under conditions still unknown is produced directly as much by the surface epithelial cells as by the cardiac or pyloric glands.

The author's opinion is that under certain physical conditions soluble mucus may be secreted directly in fluid form by the surface epithelial cells and cardiac or pyloric glands, and a part of it becomes visible mucus and peptide, and that in pathological conditions such as secondary gastritis accompanying gastric cancer soluble mucus secretion is increased so that peptide content is increased in acid cancer and protein precipitable by sulfosalicylic acid is increased in anacid cancer.

Mucoprotein and mucoprotease (GLASS) are beyond the scope of this paper.

SUMMARY

i) A peptide was present in human gastric juice particularly in acid gastric juice, and gives a polarographic protein wave. Most of it was present in the methanol supernatant fluid and dialysate.

ii) This peptide was closely related to breakdown products of protein precipitable by sulfosalicylic acid by the enzymatic action of pepsin.

iii) In most of the acid gastric cancer cases this peptide was increased. Markedly increased cases were exclusively gastric cancer.

iv) A protein precipitable by sulfosalicylic acid was only present in anacid gastric juices, and gave a typical protein double wave polarographically.

v) In most anacid gastric cancer cases this protein was increased. When it was markedly increased, gastric cancer was always present.

vi) In cases with severe atrophic gastritis and most advanced anacid cancers the protein content in gastric juice was slight.

vii) The frequency of achlorhydria in gastric cancer was 54.4%.

viii) The frequency of positive benzidine reaction was about 60% in gastric cancer and about 20% in non-cancer.

ix) When contamination with blood was not macroscopic, it had no influence on the polarographic protein wave, even when the benzidine reaction was positive.

x) Bile had a lower polarographic activity and cobalt maximum than gastric protein had, and had different wave forms from gastric protein.

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和 文 抄 録

胃 液 内 蛋 白 の 研 究

第 1 報 胃疾患特に胃癌における胃液内蛋白のポーラログラフ的研究

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覧 守

胃癌の発生と云う病理組織学的な変化に伴つて、胃液内の蛋白像が如何に変化するかをポーラログラフ蛋白波を用いて、胃癌114例を含む283例の胃液について検索し次の成績を得た。

1) 有酸胃液内には透析性で、メタノールの上清に移行する Peptide が常に存在する。

2) 有酸の胃癌胃液内にはこの Peptide の増量しているものが多く、特に35mm以上の高い蛋白波高を示したものは総て胃癌例であつた。

3) 一方無酸胃液内には Peptide は少なく、ズルホサルチル酸で沈澱する蛋白が多く存在する。

4) 無酸の胃癌胃液内にはこの蛋白の増量しているものが多く、特に30mm以上の高い蛋白波高を示したものは総て胃癌例であつた。

5) 有酸胃液内に存在する Peptide はズルホサルチル酸で沈澱する蛋白がペプシンによつて消化されて生ずる。

6) 高度に進行した胃癌例及び高度の萎縮性胃炎例

では極めて低い蛋白波高を示した。

7) 胃癌114例中胃液が無酸であつたものは54.4%であつた。

8) 胃液内の潜血反応陽性率は胃癌群では60%、非癌群では20%であつた。

9) 胃液に血液が混入すると蛋白波は高まるが、潜血反応陽性程度の軽度のものでは波高には何等の影響を及ぼさなかつた。

10) 胆汁はポーラログラフ上活性度が低く、コバルト極大の抑制力が強く、胃液蛋白とは異つた特異な波形を示した。

以上の実験結果から、胃癌の胃液内には、非特異的ではあるが或種の粗大蛋白が多量に分泌され、胃液が有酸の場合には Pepsin によつて消化されて Peptide となり、これが増量を誘起し、無酸の場合にはズルホサルチル酸で沈澱する蛋白の増量となつて現れるものと考えられる。