Passage of Serum Albumin into the Stomach

Its Detection by Paper Electrophoresis of Gastric Juice in Protein-Losing Gastropathies and Gastric Cancer

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MUCH CLINICAL INTEREST currently centers about the pathological syndromes attributable to loss of protein into the stomach,[1, 2] small intestine,[2-8] and colon.[9] These syndromes have been described under the names of hypercatabolic[2, 3] and anabolic[5] hypoproteinemias, exudative enteropathies,[4] and protein-losing gastroenteropathies.[10] They have been diagnosed by the presence, chemically detected, of albumin in the gastric juice,[1] by the accelerated turnover in the body of injected I^{131}-labeled albumin[1-3, 5, 8] or, finally, by the increased permeability of the gastrointestinal mucosa to injected I^{131}-labeled Polyvinyl Pyrrolidone (PVP).[4, 7]

A protein-losing gastropathy can be readily diagnosed, also, by paper electrophoresis of the gastric juice.[11] A case of Menetrier's disease (hypoproteinemia associated with giant hypertrophy of the gastric mucosa) reported in 1957 by Citrin *et al.* gave us the first opportunity to study the gastric secretion of a patient who had a proved case of protein-losing gastropathy by the electrophoretic method.

The massive leakage of serum albumin into the gastric juice of this patient was demonstrated by the presence of an appreciable amount of protein-bound I^{131}-labeled albumin and the precipitability of this radioactive material by trichloroacetic acid. When this gastric juice was sent to us through the kindness of Citrin and his co-workers, we

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found, on paper electrophoresis,[1] that it contained a large, heavily stained protein band at the anodic side of the tracing, which differed markedly in mobility and staining properties from those of gastric mucoproteins but was similar to that of serum albumin.

In 1958, at the World Congress of Gastroenterology, we reported[11] 6 other cases where electrophoretic tracings of the gastric juice showed the massive leakage of an albumin-like material into the gastric lumen. These cases included a second case of Menetrier's disease, 3 of atrophic gastritis (2 associated with pernicious anemia), and 2 of gastric cancer. Since then we have collected several additional cases, normal and pathological, where passage of serum albumin into the stomach has been demonstrated by paper electrophoresis of the gastric juice. Some of this material, including several cancer cases, has been presented in a preliminary form at a meeting of the Federated Societies.[12] Similar findings in 5 of 9 cases of gastric cancer have recently been reported by Schwartz and Jarnum.[13] We propose here to add further data gathered since that time and to present all our material in a more detailed and comprehensive form.

METHOD

Paper electrophoresis of the gastric juice was performed by a method described earlier:[14, 15] centrifuged, dialyzed, and freeze-dried gastric juice is dissolved at 2% concentration in borate buffer of *p*H 9.0 and ionic strength 0.24, and 0.05 ml. of this solution, containing 1 mg. of dry material, is applied to Whatman No. 1 paper and run in a vertical Spinco cell against the borate buffer at 0.4 mAmp./cm. and 120 v. for 5½ hr. The oven-dried strips are then stained with a saturated solution of amido black 10B, washed with several changes of methanol and acetic acid, then air dried, scanned, traced, and integrated in the Analytrol (Spinco) with a B2 cam and a 575 mμ filter. The other electrophoretic strips are, in addition, stained with SF light green[16] and periodic acid-Schiff (PAS)[17] stains, then run in the Analytrol, using a 620 mμ filter for the SF light green stain and a 550 mμ filter for the PAS stain.

ELECTROPHORETIC PATTERN OF NORMAL GASTRIC JUICE

The normal electrophoretic pattern of dialyzed and lyophilized acid gastric secretion collected after histamine stimulation (Fig. 1) consists of 8–11 components (4–5 anodic and 4–6 cathodic).[15, 18]

The leading anodic component of the tracing, as shown by elution studies,[19] corresponds to pepsin, and for this reason we called it peak P. The other anodic boundaries, named M_1, M_2, M_3 (this often subdivided into 2 components, M_{3a} and M_{3b}), and M_4 (which travels by endosmosis

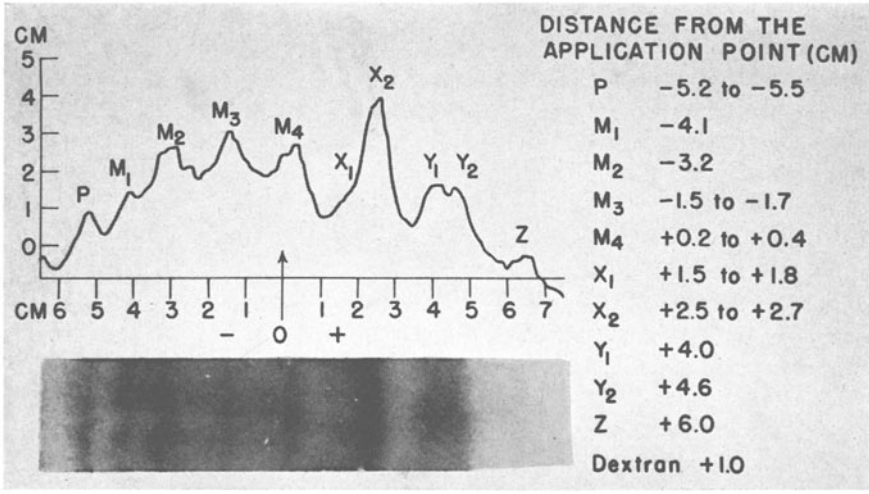

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Fig. 1. Paper electrophoretic pattern of a pool of normal gastric juices. (Amido black stain)

to the cathode), take up amido black 10B, SF light green, and PAS stains. On elution, these materials were found to contain both proteins and carbohydrates.[20] They represent mucoproteins, which accounts for our use of the letter M to designate them.

On the cathodic side, there are 4 or 5 bands which, for initial lack of knowledge as to their nature, we named X, Y, and Z.[15] Materials X and Y often consist of 2 subcomponents, namely, X_1, X_2, Y_1, and Y_2. Of these, only component X_1 takes up protein stain and some carbohydrate stain, whereas materials X_2, Y_1, Y_2, and Z take up neither protein nor carbohydrate stain and, on elution, contain no protein or carbohydrate material. The latter components represent large molecular polypeptides that are not readily dialyzable, contain organic bases, and are derived mainly from the peptic degradation of other large molecular components of the gastric juice.[20, 21]

In Fig. 2 the electrophoretic pattern of normal gastric juice is compared with that of serum when run by the same technic. Serum albumin has an electrophoretic mobility midway between that of gastric components M_1 and M_2. $Alpha_1$ and $alpha_2$ globulins have mobilities similar to those of gastric materials M_2 and M_{3a}; serum gamma globulin has a mobility similar to that of gastric component M_4. Comparative elution studies of mucoproteins of serum and gastric juice have shown that serum mucoproteins differ from gastric mucoproteins by a much higher sialic acid, and a much lower fucose (methylpentose), content.[20]

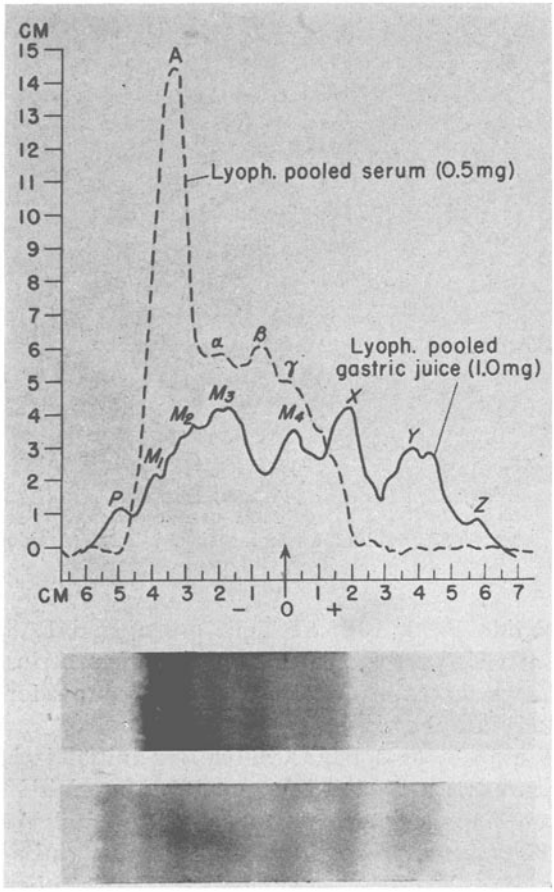

Fig. 2. Comparison of paper electrophoretic patterns of normal pooled blood serum and normal gastric juice, both processed by the same method and stained with amido black stain.

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DETECTION OF SERUM ALBUMIN IN GASTRIC JUICE BY PAPER ELECTROPHORESIS

The staining properties of albumin differ from those of pepsin and gastric mucoproteins. This is illustrated in Fig. 3, which shows the electrophoregrams of 4 patients who had serum albumin in the gastric juice. The strong uptake of both amido black and SF light green stains, and the lack of, or mere trace uptake of PAS stain, distinguishes albumin from any other of the gastric mucoproteins.

As shown in Fig. 4, when serum albumin is added to anacid gastric juice that contains serum albumin, it becomes superimposed over the anodic band in question, increasing it markedly. It has a mobility midway between that of gastric mucoproteins M_1 and M_2. If the total length of the partition is 11–12 cm., it is localized 3.5 cm. toward the anode from the point of application.

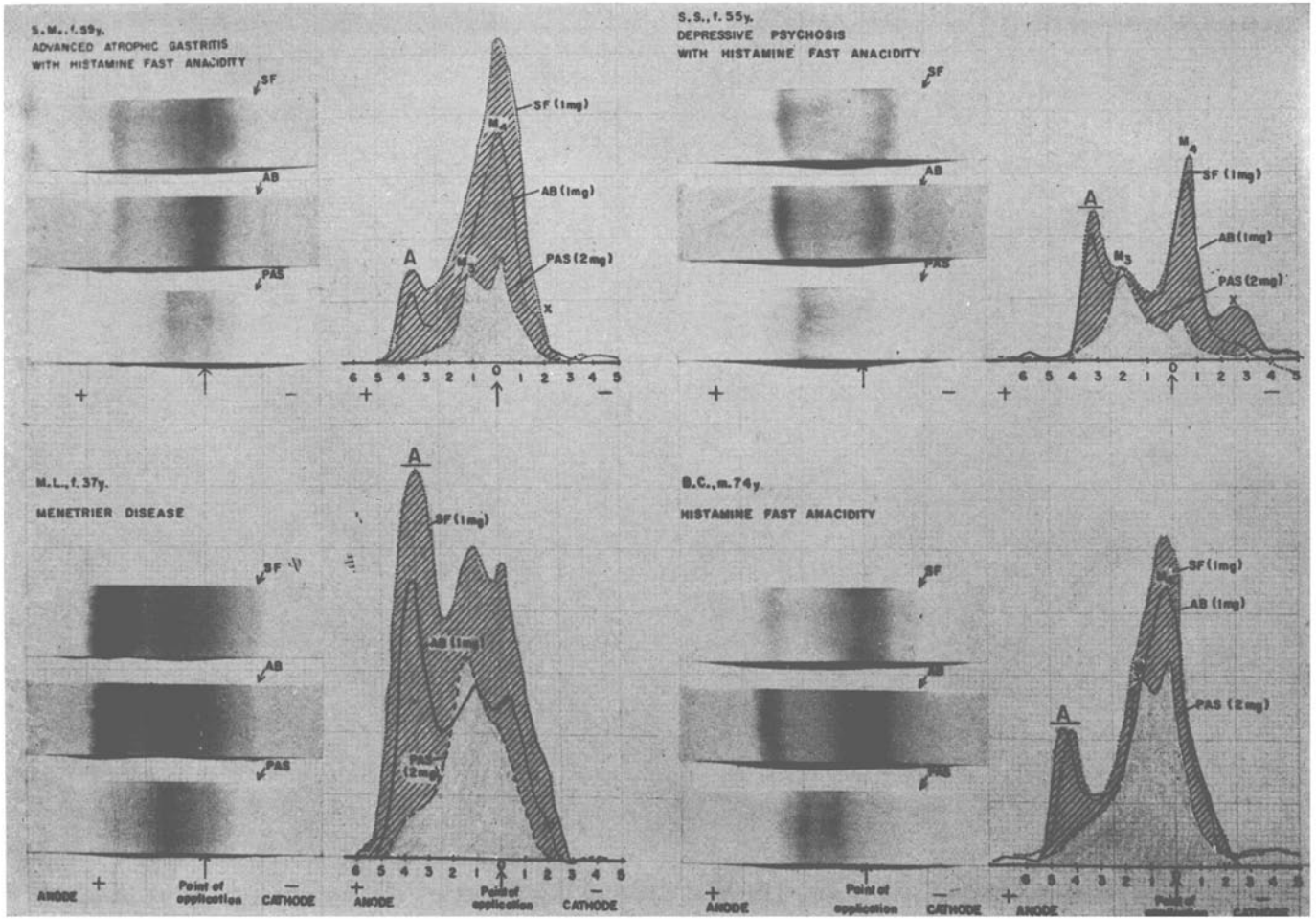

Fig. 3. Albumin in gastric electrophoregrams of 4 patients with histamine-fast anacidity. (Amido black 10B, SF light green, and PAS stains)

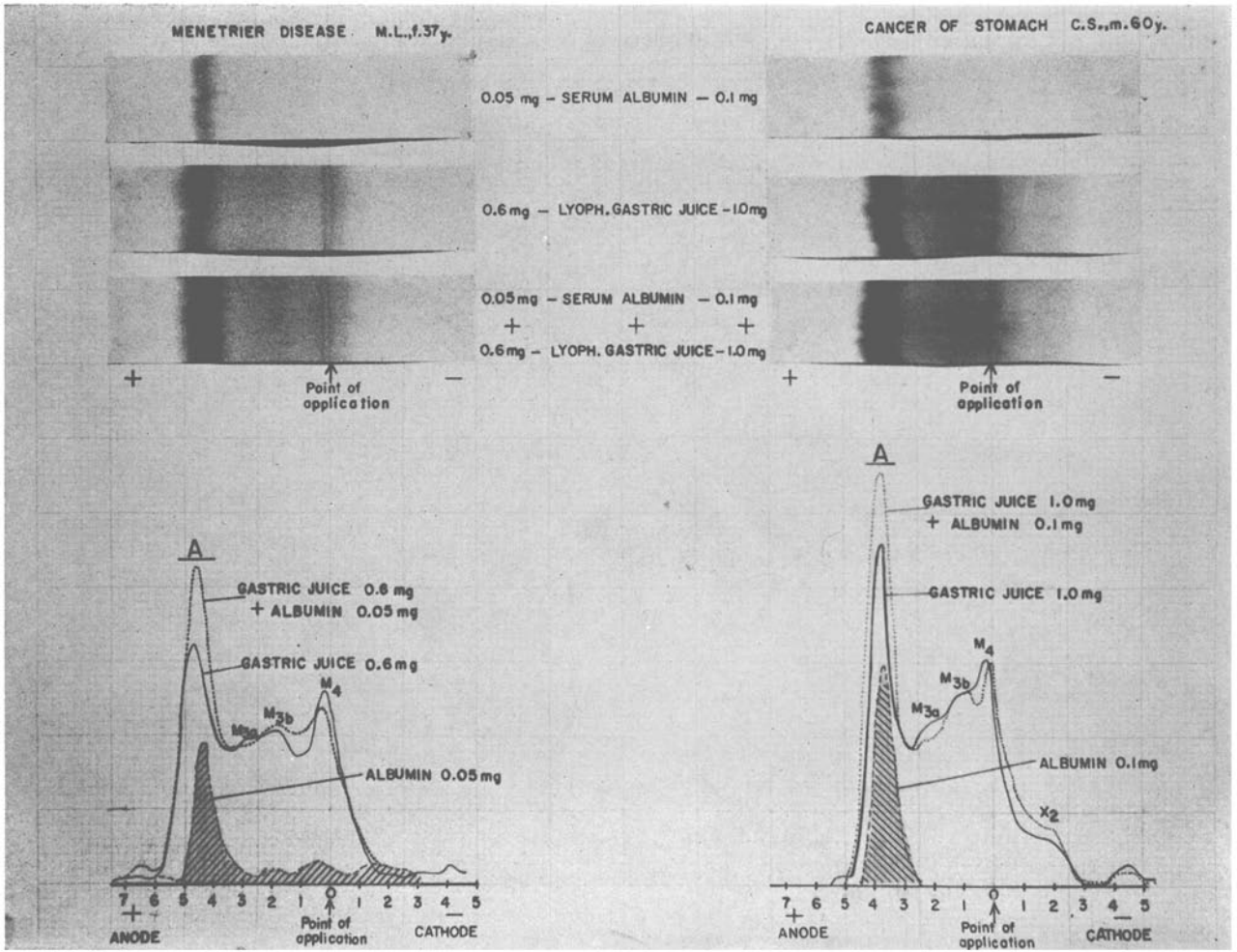

Fig. 4. Serum albumin added to gastric juices of patients with protein-losing gastropathies. From *Current Gastroenterology* (Amido black stain)

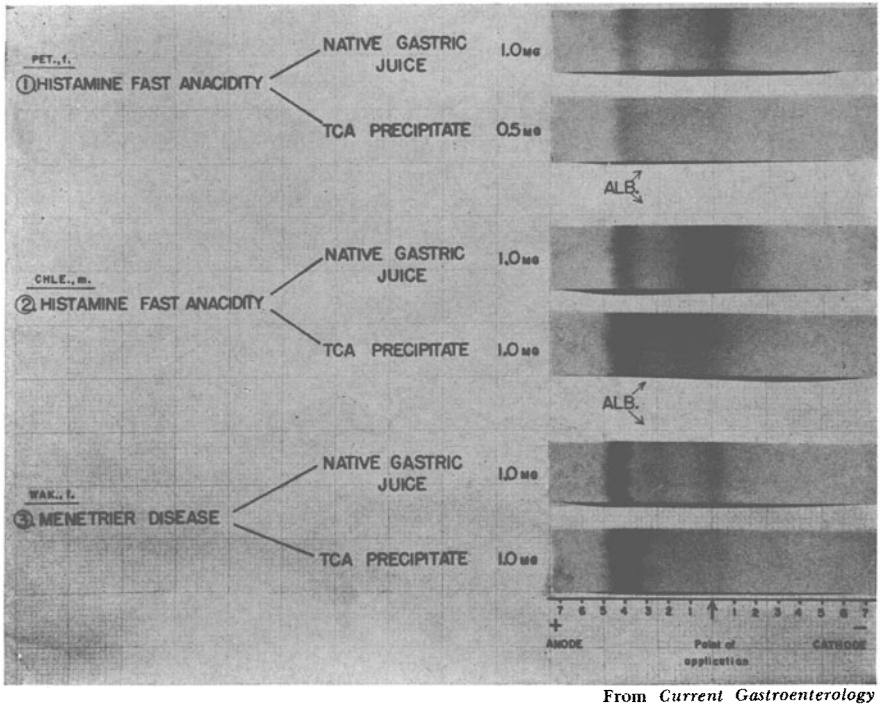

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Fig. 5. Trichloroacetic acid precipitation of serum albumin from three gastric juices. (Amido black stain)

This material is precipitable by trichloroacetic acid, and the precipitate is soluble in 95% alcohol or 80% acetone, a characteristic feature of serum albumin. Figure 5 shows that the electrophoretic mobilities of albumin-like material from three native gastric juices were identical to those of trichloroacetic acid precipitate obtained from the same sources. This confirms the serum albumin nature of the albumin-like material in the gastric juice.

We found paper electrophoretic evidence of the presence of serum albumin in gastric juices collected from 40 of a total of 400 patients with and without gastric pathology. All 40 juices were anacid, on Toepfer titration, and their *p*H was above 3. In acid gastric juice, the albumin, if present, becomes digested by pepsin and no longer shows as a typical anodic component with the mobility and staining properties of serum albumin.

PEPTIC DEGRADATION OF SERUM ALBUMIN

Figure 6 shows a prominent serum albumin component in the fasting anacid gastric juice of a patient with gallstone disease. After acid and

pepsin secretion had been stimulated by histamine injection, the albumin component decreased markedly and was recognizable on the Analytrol tracing but not on the stained strip.

As Ohara has shown in our laboratory,[21] when serum albumin is incubated with pepsin for 15 min., the albumin band is markedly reduced in size and several additional bands are formed, some localized in the central anodic area and some at the cathodic side of the electrophoretic partition (Fig. 7). After the serum albumin has been digested

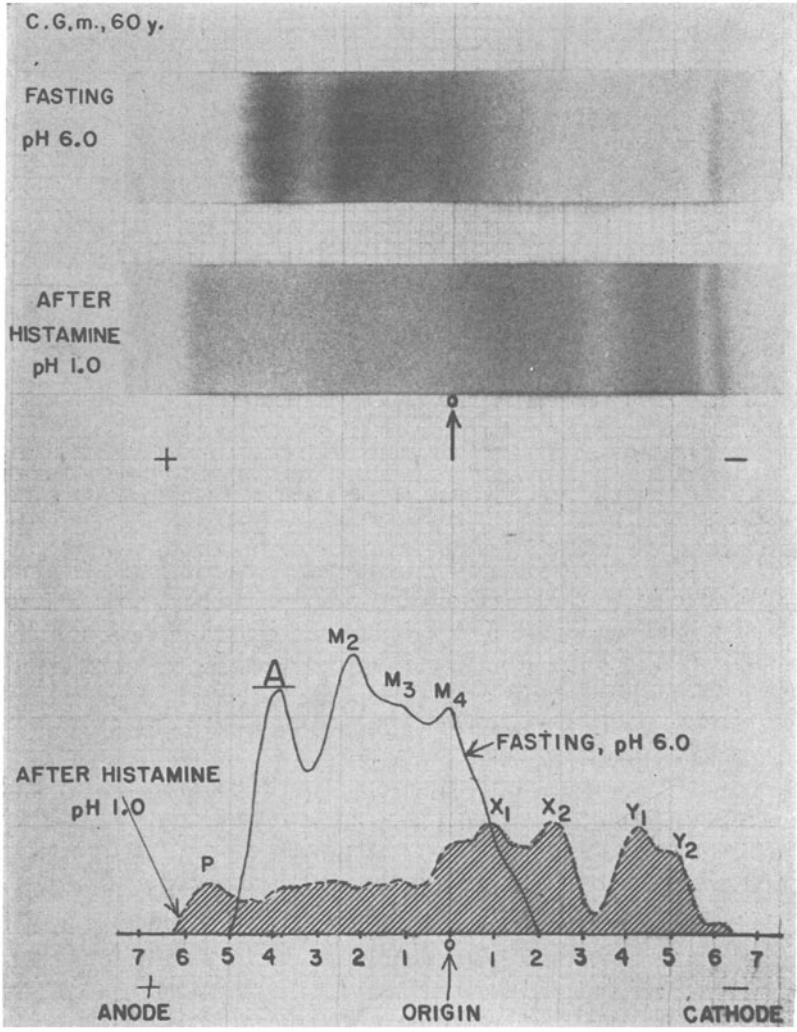

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Fig. 6. Disappearance of albumin band from electrophoretic pattern of gastric juice after stimulation of secretion of acid and pepsin by histamine. (Amido black stain)

for 60 min., the degradation becomes still more pronounced, the albumin peak declines still further, and new components that have the electrophoretic mobilities of components X_2, Y_1, and Y_2 appear prominently at the cathodic side of the tracing.[21] This sequence in the degradation of albumin is shown in Fig. 7.

The same process occurs when serum albumin is incubated with human gastric juice that contains acid and pepsin. As shown in Fig. 8, most of the albumin material is digested within 15 min. and forms degradation products that have mobilities similar to those of cathodic components X_2, Y_1, and Y_2. The typically located albumin peak decreases markedly, although it is still detectable on the Analytrol tracing.

When albumin is incubated with anacid gastric juice of *p*H 3.0, which shows no peptic activity at *p*H 1.5, no degradation products of the type just described appear. Under these circumstances, the albumin component now blurs, widens, and tends to spread toward the anode, and another, more negatively charged, albumin derivative appears to be formed.

The gastric juices of patients with superficial gastritis, gastric hyperacidity, or duodenal ulcer, which have high acid and pepsin concentrations, show the presence of cathodic components X_2, Y_1, and Y_2.

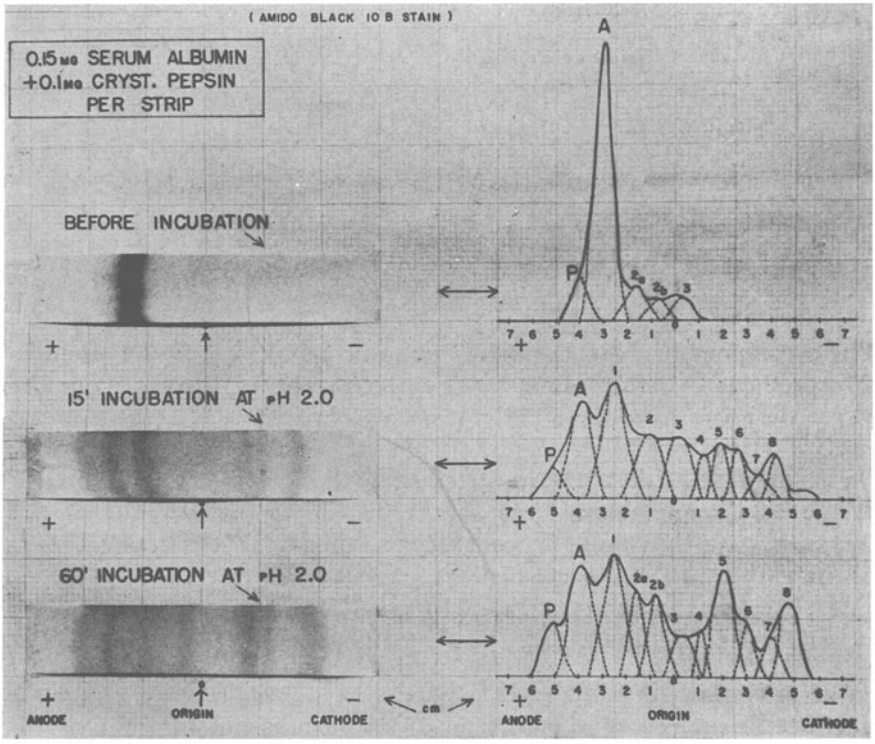

Fig. 7. Peptic digestion of serum albumin. (Amido black stain)

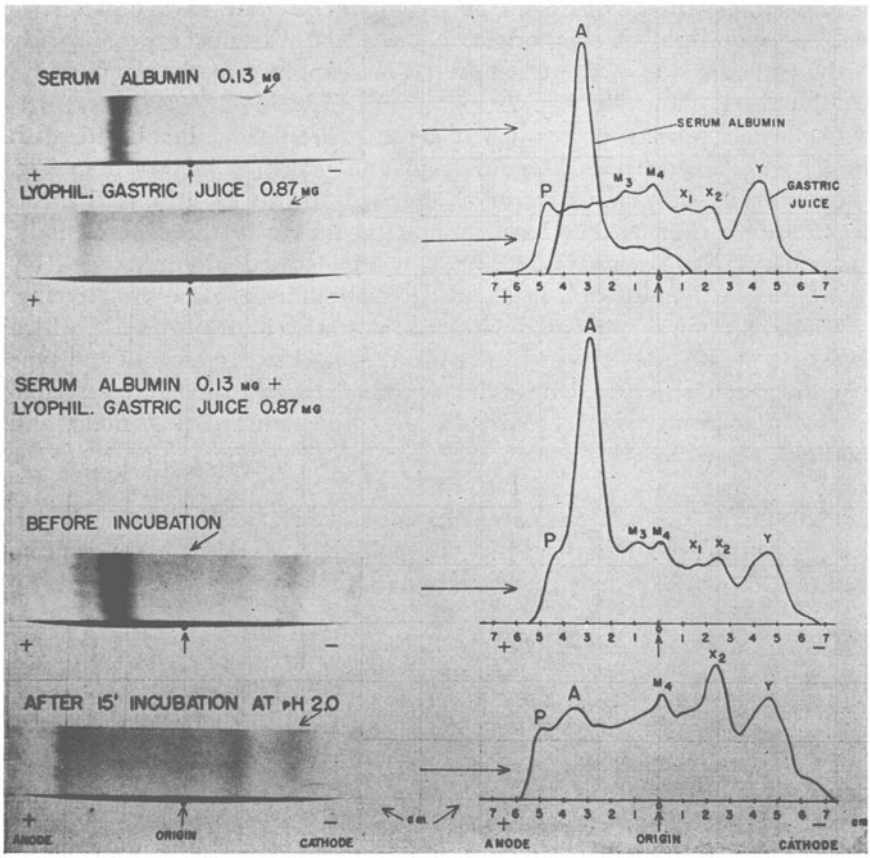

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Fig. 8. Paper electrophoretic patterns of serum albumin before and after incubation with normal gastric juice. (Amido black stain)

These components have the electrophoretic mobilities of the degradation products of serum albumin. Fourteen strips of the same number of gastric juices from these patients are shown in Fig. 9.

With Hitzelberger,[22] we found a similar situation in patients with rheumatoid arthritis who were treated with massive doses of prednisolone or dexamethasone. As shown in Fig. 10, as a result of this treatment, massive cathodic components appear in electrophoregrams of the gastric juices of some of these patients.

QUANTITATION OF SERUM ALBUMIN IN GASTRIC JUICE BY PAPER ELECTROPHORESIS

We attempted by paper electrophoresis to gain more insight into the quantitative aspects of the leakage of serum albumin into the gastric juice, under both normal and pathological conditions.

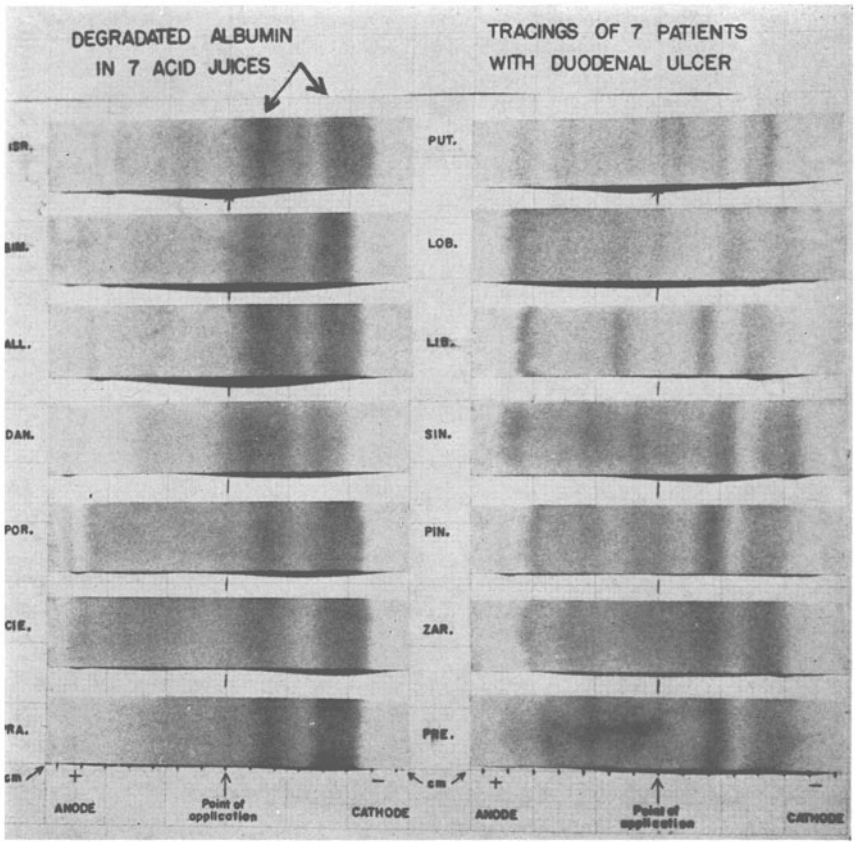

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Fig. 9. Paper electrophoretic tracings of gastric juice of 7 patients with duodenal ulcer and 7 patients with gastric hypersecretion, collected after histamine stimulation. (Amido black stain)

Method

Human serum albumin was submitted to paper electrophoresis under conditions similar to those set for the electrophoresis of gastric juice, 0.02 ml. of serum albumin in borate buffer being applied to paper strips at concentrations increasing in range from 0.25 to 2.0%, and then run on electrophoresis, as described above for gastric juice. After the strips had been scanned and traced, the areas under the albumin components were drawn with the use of gaussian curves. The surface of the curves was then integrated and calculated in integration units with the use of Analytrol. From these figures, the calibration curve of serum albumin was contructed, as reproduced in Fig. 11. The Analytrol gain was set for 5.0 cm. for this curve, owing to our use of a Spinco B-2 cam uncorrected for serum albumin. This calibration curve proved

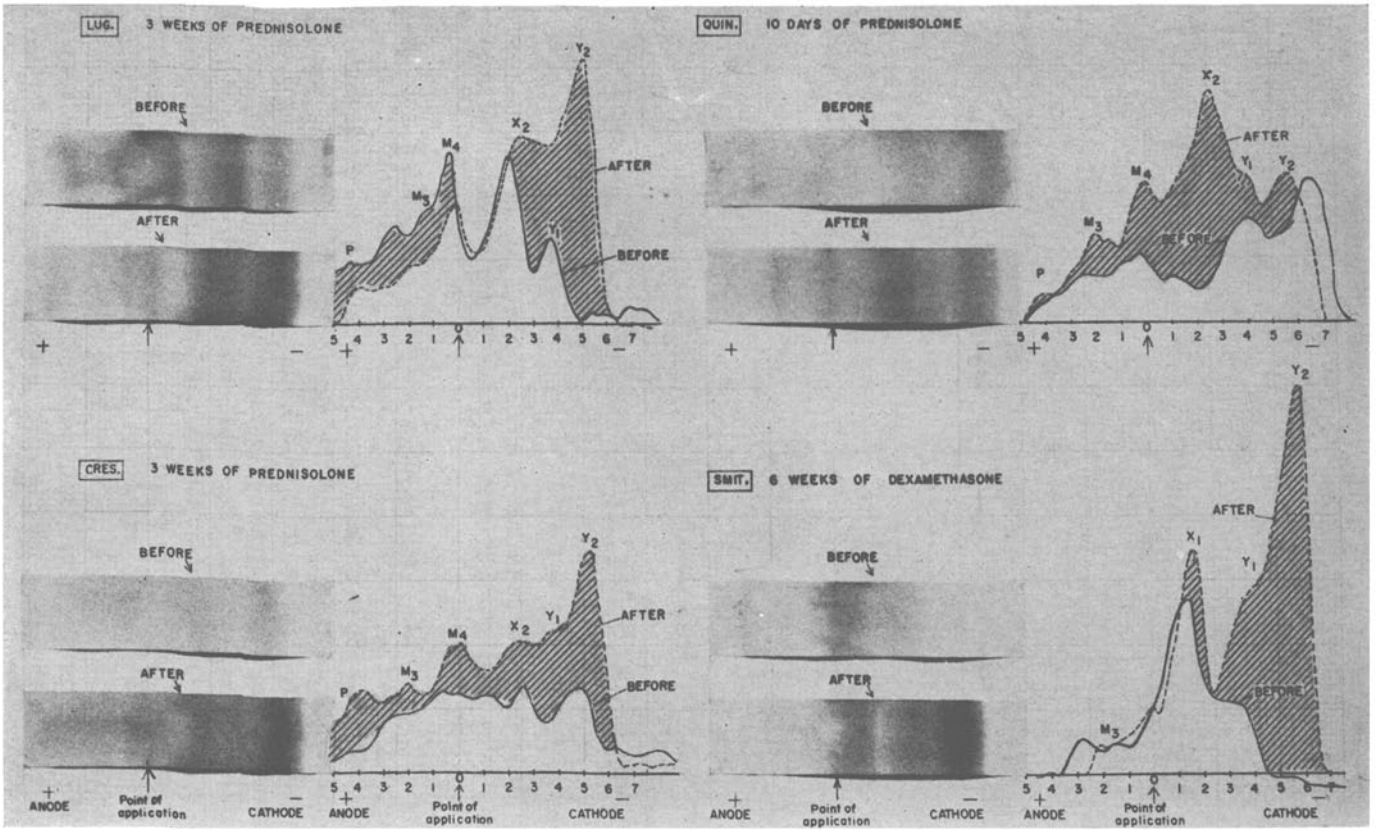

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Fig. 10. Formation of heavy cathodic bands in electrophoretic tracings of fasting gastric juices of 4 patients with rheumatoid arthritis, after treatment with corticosteroids. (Amido black stain)

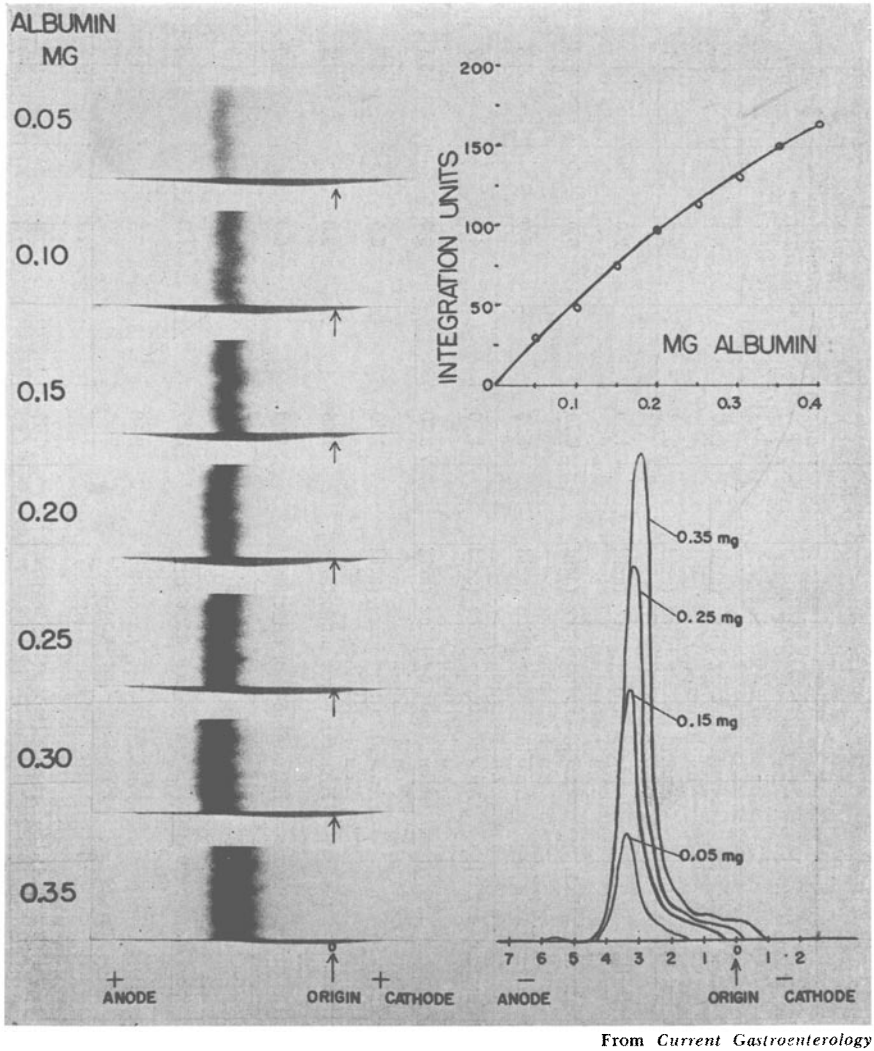

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Fig. 11. Quantitation of albumin by paper electrophoresis. Calibration curve of albumin. (Amido black stain)

to have a parabolic shape and to show a flattening at the higher concentrations. With the use of gaussian curves, we read the integration figures corresponding to the albumin component of the gastric juice studied, then interpolated it on the calibration curve, and read from it the serum albumin content in milligrams per strip. Knowing the amount of lyophilized gastric juice applied to the strip and the yield

TABLE 1. ALBUMIN CONCENTRATION IN THE ANACID GASTRIC JUICES OF 40 PERSONS

No.	*Name*	*Age, Sex*	*Diagnosis*	*Gastric secretory pattern*	*Nondialyzable solids in gastric juice (mg./ml.)*	*Serum albumin, mg. per 100 mg. nondialyzable solids of gastric juice*	*Serum albumin, mg. per 100 ml. native gastric juice*
1.	Got.	M, 53	Gallstone disease	Anacid fasting juice (acid on histamine)	1.00	5.5	5.5
2.	Las.	M, 47	Postcholecystectomy	Anacid fasting juice (acid on histamine)	0.53	2.0	1.1
3.	Hea.	M, 67	Diverticulosis coli	Anacid fasting juice (acid on histamine)	1.89	2.0	3.8
4.	Riv.	M, 50	Chronic alcoholism	Anacid fasting juice (acid on histamine)	0.76	5.0	3.8
5.	Spr.	F, 54	Depressive psychosis	Anacid fasting juice (acid on histamine)	1.23	8.0	9.8
6.	Bla.	F, 61	Rheumatoid arthritis	Anacid fasting juice (acid on histamine)	1.70	4.5	7.7
7	Cru.	F, 27	Rheumatoid arthritis	Anacid fasting juice (acid on histamine)	2.70	3.0	8.1
8.	Smi.	F, 68	Osteoarthritis	Anacid fasting juice (acid on histamine)	1.40	2.5	3.5
9.	Sch.	F, 60	Gastric ulcer; postgastrectomy	Histamine-fast anacidity	1.46	3.0	4.4
10.	Sul.	M, 72	Gastric ulcer; postgastrectomy	Histamine-fast anacidity	1.00	6.0	6.0
11.	Ada.	M, 50	Duodenal ulcer; postgastrectomy	Histamine-fast anacidity	2.53	3.5	8.9
12	Kru.	F, 69	Coronary insufficiency; atrophic gastritis	Histamine-fast anacidity	2.25	11.0	24.8
13.	Chl.	M, 70	Hypertrophy prostate	Histamine-fast anacidity	2.10	7.5	15.8
14.	Pet.	F, 57	Large gastric rugae	Histamine-fast anacidity	2.63	5.0	13.2
15.	Kie.	M, 55	Advanced atrophic gastritis	Histamine-fast anacidity	1.62	4.5	7.2
16.	Mar.	F, 57	Advanced atrophic gastritis	Histamine-fast anacidity	0.77	12.0	9.2
					1.87	7.0	13.7
					1.00	6.0	6.0

17.	Tho.	F, 77	Advanced atrophic gastritis	Histamine-fast	anacidity	1.60	12.5	20.0
18.	Wak.	F, 60	Advanced atrophic gastritis	Histamine-fast	anacidity	1.44	14.0	20.1
19.	Mea.	M, 82	Pernicious anemia	Histamine-fast	anacidity	3.90	15.0	58.5
20.	Mar.	M, 65	Tropical sprue	Histamine-fast	anacidity		23.0	
21.	Has.	M, 74	Rheumatoid arthritis (on cortisone)	Histamine-fast	anacidity	1.80	37.0	66.0
22.	Lew.	F, 54	Rheumatoid arthritis (on cortisone)	Histamine-fast	anacidity	3.0	28.0	84.0
23.	Lop.	M, 60	Menetrier's disease	Histamine-fast	anacidity	2.14	24.0	51.4
24.	Mon.	M, 71	Menetrier's disease	Histamine-fast	anacidity		15.0	
25.	Lyn.	F, 37	Menetrier's disease	Histamine-fast	anacidity	3.1	26.5	88.2
						4.5	25.0	112.5
26.	Pru.	M, 63	Menetrier's disease	Histamine-fast	anacidity	2.8	36.0	100.8
27.	Bur.		Menetrier's disease	Histamine-fast	anacidity	7.4	24.0	177.6
28.	Cos.	M, 39	Lymphosarcoma of stomach	Histamine-fast	anacidity	1.80	12.5	22.5
29.	Ros.	F, 67	Gastric carcinoma	Histamine-fast	anacidity	1.44	4.5	6.6
30.	Rei.	F, 64	Gastric carcinoma	Histamine-fast	anacidity	9.0	4.0	36.0
31.	Nab.		Gastric carcinoma	Histamine-fast	anacidity	1.57	15.0	23.4
32.	Kor.	M	Gastric carcinoma	Histamine-fast	anacidity	1.12	17.5	19.6
33.	deW.	F, 65	Gastric carcinoma	Histamine-fast	anacidity	2.0	17.0	34.0
34.	Sch.	M, 50	Gastric carcinoma	Histamine-fast	anacidity	4.90	31.0	152.0
35.	Pet.	F	Gastric carcinoma	Histamine-fast	anacidity	2.0	36.0	72.0
36.	Nat.		Gastric carcinoma	Histamine-fast	anacidity	6.50	42.0	273.0
37.	Fie.		Gastric carcinoma	Histamine-fast	anacidity	9.10	53.0	482.0
38.	Roe.	F	Gastric carcinoma	Histamine-fast	anacidity	3.40	60.0	204.0
39.	Kru.	M	Gastric carcinoma	Histamine-fast	anacidity	2.60	60.0	156.0
40.	McS.	F	Gastric carcinoma	Histamine-fast	anacidity	3.10	63.0	195.0

of lyophilized powder per 100 ml. of native gastric juice, we calculated the serum albumin concentration in (1) milligram per weight of nondialyzable solids and (2) in milligrams per millilter of gastric juice, according to the equation:

$$A_d = \frac{A_c \times 100}{w} \tag{1}$$

in which A_d is the content of albumin in milligrams per 100 milligrams of dry weight of nondialyzable solids in the gastric juice; A_c is the content, in milligrams, of albumin on the strip as read from the calibration curve; and w is the weight, in milligrams, of dialyzed and lyophilized gastric juice applied to the strip, and the equation:

$$A_n = \frac{A_c \times 100 \times y}{w} \tag{2}$$

in which A_n is the concentration of albumin in milligrams per 100 ml. of native gastric juice, A_c and w are as defined above, and y is the yield, in milligrams of dry, nondialyzable gastric solids per milliliter of native gastric juice.

In the example $A_c = 0.15$ mg., $w = 1$ mg., and $y = 1.6$ mg./ml.

$$A_n = \frac{0.15 \times 100 \times 1.6}{1} = 24 \text{ mg./100 ml.} \tag{3}$$

Results

In Table 1 the results of the quantitation of serum albumin in 40 anacid gastric juices containing serum albumin are listed. We have classified our material somewhat arbitrarily into 2 groups: (1) Patients who have a moderate or small content of albumin in the gastric juice (below 15% w/w of nondialyzable solids), and (2) Patients who show a massive serum albumin leakage into the gastric juice (15-63% w/w of nondialyzable solids).

Data on 21 anacid gastric juices with small or moderate amounts of serum albumin are listed in Table 1, and those for 7 are shown in Fig. 12. Eight of the 21 juices (No. 1–8) represent collections from subjects whose gastric juice contained no free acid or pepsin under fasting conditions, but did so after histamine stimulation. The concentration of serum albumin in these 8 juices varied from 2–8% w/w of the nondialyzable solids. After reconversion of the data to values for native juice, the albumin concentration in each patient was below 10 mg./100 ml. of gastric juice—i.e., below 0.01%.

The concentration of serum albumin in the fasting gastric juices of the remaining 13 patients with histamine-fast anacidity is also listed

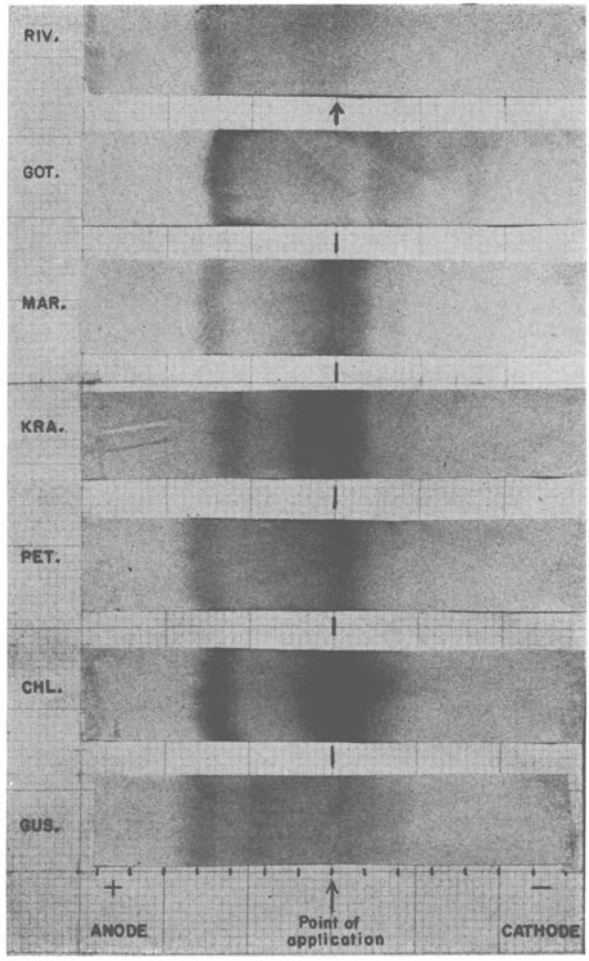

Fig. 12. Serum albumin in paper electrophoretic patterns of 7 anacid gastric juices. (Amido black stain)

in Table 1 (No. 9–18, 28–30). Albumin in this group ranged from 3 to 14% w/w of the nondialyzable solids of gastric juice. After reconversion of the data to values for native gastric juice, the concentration of serum albumin here was from 4.4 to 24.8 mg./100 ml. In Fig. 13, the electrophoretic tracings of the gastric juices of 3 of these patients are compared with that of a patient with duodenal ulcer.

In 19 patients we found a massive leakage of serum albumin into the gastric lumen, as listed in Table 1. These include 10 patients who had gastric cancer (No. 31–40), one of which (No. 31) occurred after pernicious anemia; 1 patient with pernicious anemia (No. 19); 1 patient who had sprue with histamine-fast anacidity (No. 20); 5 who had Menetrier's disease (No. 23–27), and 2 (No. 21 and 23), observed with

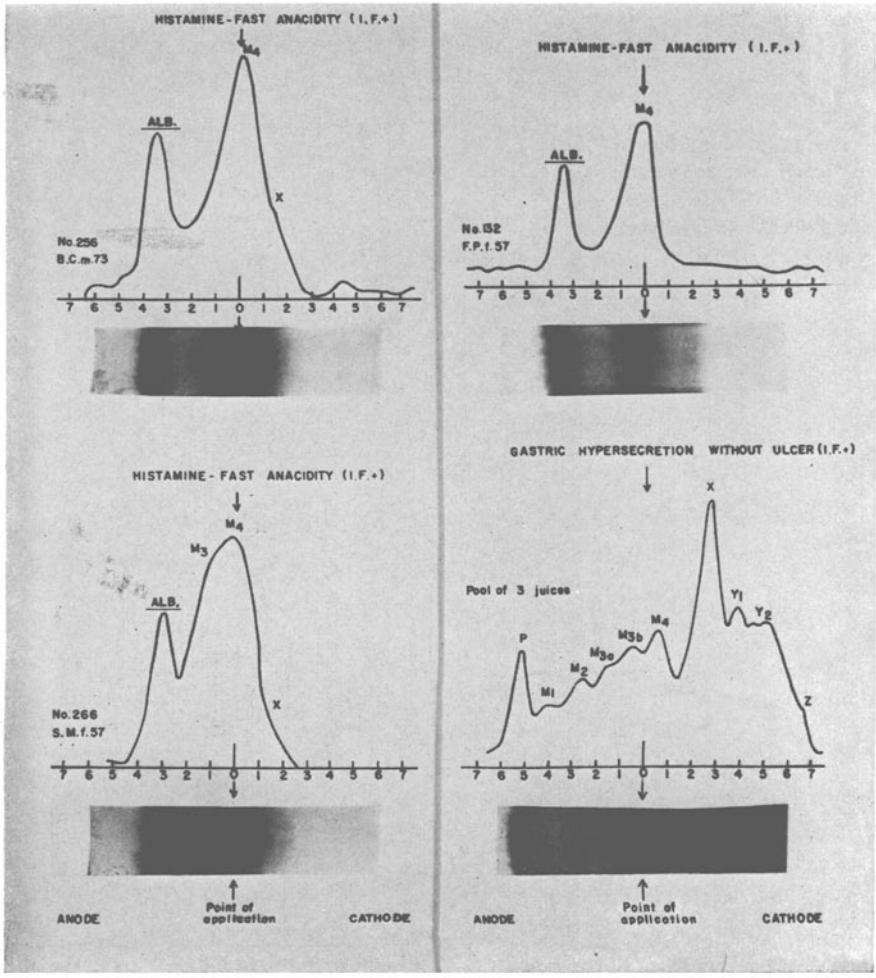

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Fig. 13. Serum albumin in electrophoretic patterns of gastric juice of 3 patients with histamine-fast anacidity. The electrophoretic pattern of a pool of three juices of patients with gastric hypersecretion is shown for comparison. (Amido black stain)

Dr. A. Hitzelberger,[22] who had rheumatoid arthritis that had been treated with large amounts of steroids. Before steroid treatment, these patients had had no serum albumin in their gastric juice.

Fourteen tracings of patients with massive albumin leakage are shown in Fig. 14, where they are compared with the tracings of 7 normal juices. The electrophoretic tracings of the 2 patients on steroid treatment are shown in Fig. 15.

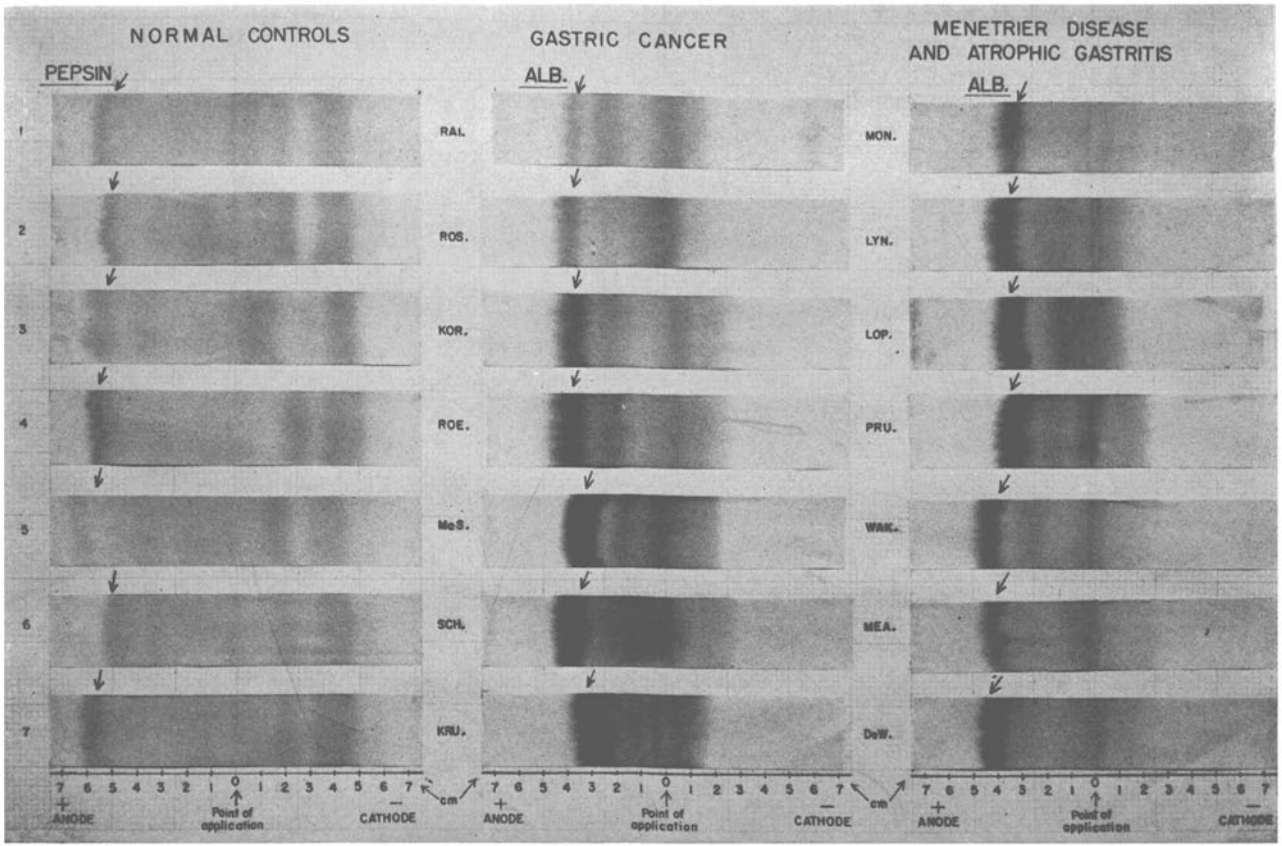

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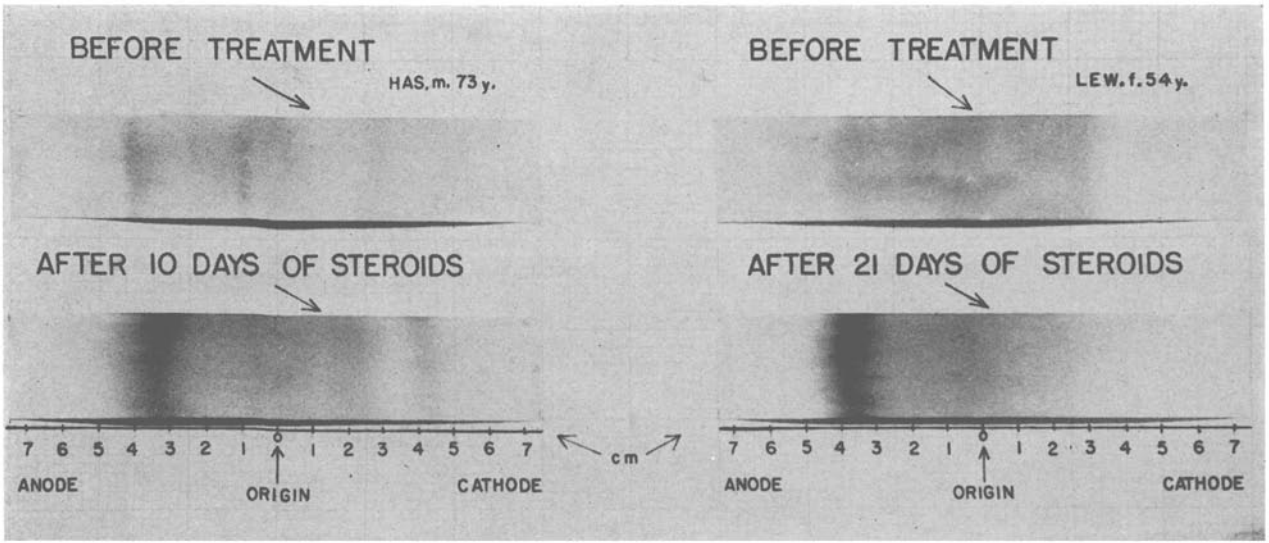
Fig. 14. Serum albumin in paper electrophoretic tracings of 14 patients with protein-losing gastropathies, including 7 with gastric cancer, 4 with Menetrier's disease (Mon., Lyn., Lop., and Pru.), 1 with advanced atrophic gastritis (Wak.), and 2 with pernicious anemia (Mea., DeW.), one of which (DeW,) had gastric cancer as a complication. The tracings of 7 normal individuals with free acid and pepsin in the gastric juice are shown for comparison. (Amido black stain)



From *Current Gastroenterology*

Fig. 15. Serum albumin in electrophoregrams of gastric juice of 2 patients with rheumatoid arthritis after steroid treatment. (Amido black stain)

The leakage of serum albumin into the gastric juice in Menetrier's disease is illustrated in Fig. 16. The location of the bands, the strong uptake of amido black 10B and SF light-green stains, and the absence or mere trace uptake of PAS stain proved to be characteristic for serum albumin (Fig. 3). During the 3 yr. since our initial study of the first such juice sent to us by Citrin *et al.*,[1] we have had an opportunity to examine an additional 4 patients having this protein-losing gastropathy. Three of these were referred to us. In each case, the gastric juice proved to be anacid and, on electrophoresis, to contain a massive serum albumin band.

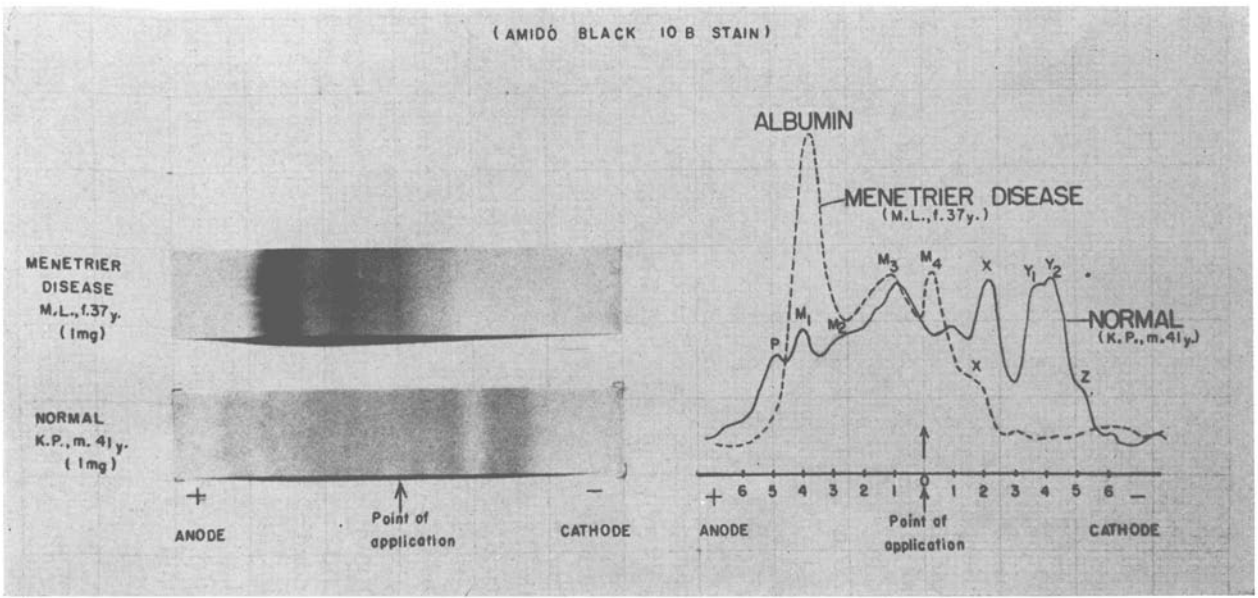
The concentration of serum albumin in the gastric juice of patients with Menetrier's disease ranged from 15.0 to 36.0 mg./100 mg. nondialyzable solids of gastric juice. After reconversion of the data to values for native gastric juice, the concentration of serum albumin was in the range of 51.4-177.6 mg./100 ml. These figures are much higher than those of the serum albumin content of anacid gastric juices of human subjects with normal gastric secretion or of patients with histamine-fast anacidity due to atrophic processes in the gastric mucosa.

Of our total of 20 patients who had gastric cancer on whom paper electrophoresis of the gastric juice was done, 8 had free gastric acid and pepsin, and 12 reported here had histamine-fast anacidity. The acid- and pepsin-containing gastric juices of 8 of our cancer patients revealed no abnormality of electrophoretic pattern and contained all the normal components, including the cathodic bands. On the other hand, in 10 of the 12 patients who had gastric cancer associated with histamine-fast anacidity, we found a massive leakage of serum albumin into the gastric juice. Patterns of some of these cases are shown in Fig. 4, 14, 17, and 18. The albumin content here ranged from 15 to 63 per cent of the nondialyzable solids, or from 19.6 to 484.0 mg. albumin per 100 ml. of native gastric juice. It should be noted that, in 6 of these 10 patients the albumin content was as high as 36 to 63 per cent of the nondialyzable solids and exceeded 150 mg./100 ml. of native gastric juice.

Ohara, in our laboratory, has demonstrated by immunoelectrophoretic technic the identity of the albumin-like material in the gastric juice with serum albumin in 1 of our patients who had Menetrier's disease and in 2 who had gastric cancer.[21]

GAMMA GLOBULIN IN GASTRIC JUICE

In several gastric juices containing serum albumin (Fig. 3, 12, and 13), we found some material situated at the cathodic side of the tracing, close to the application point, which had the characteristics of gamma



From *Current Gastroenterology*

Fig. 16. Paper electrophoretic tracings of gastric juice of a patient with Menetrier's disease and of a normal individual, after histamine stimulation. (Amido black stain)

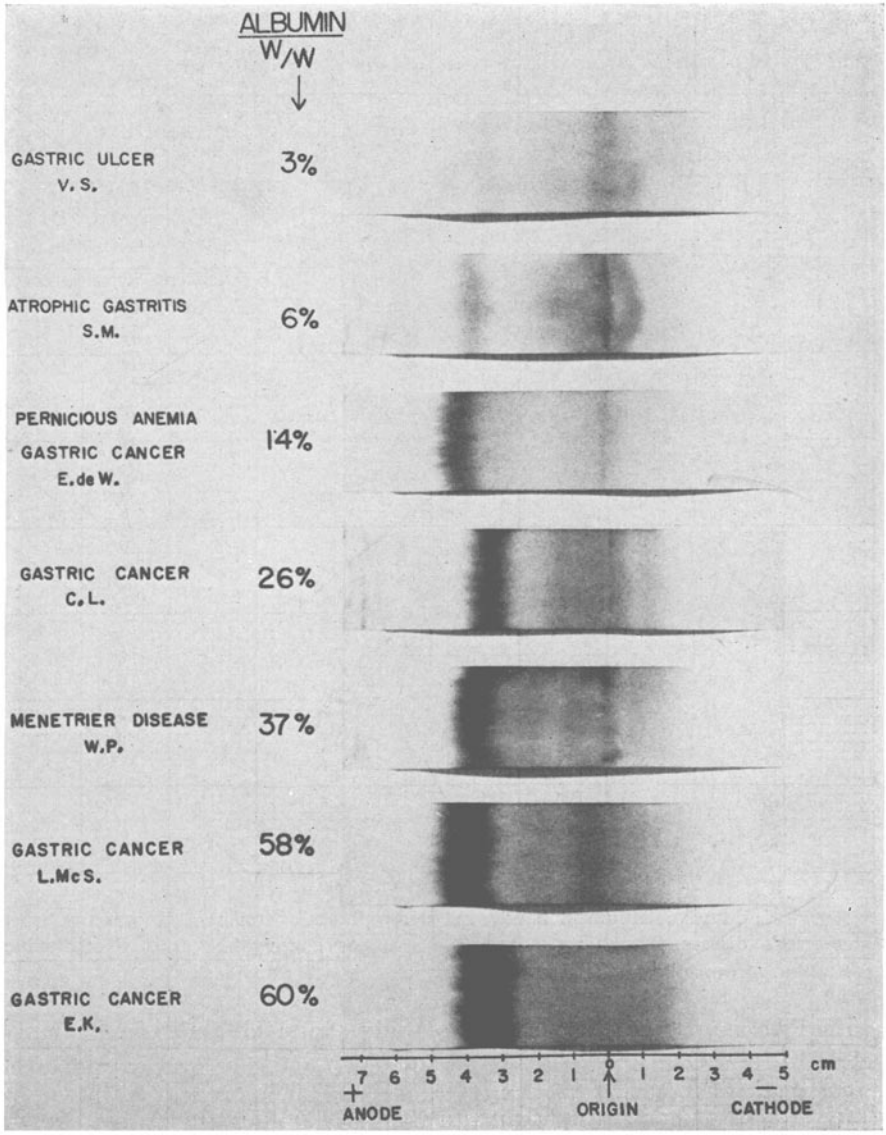


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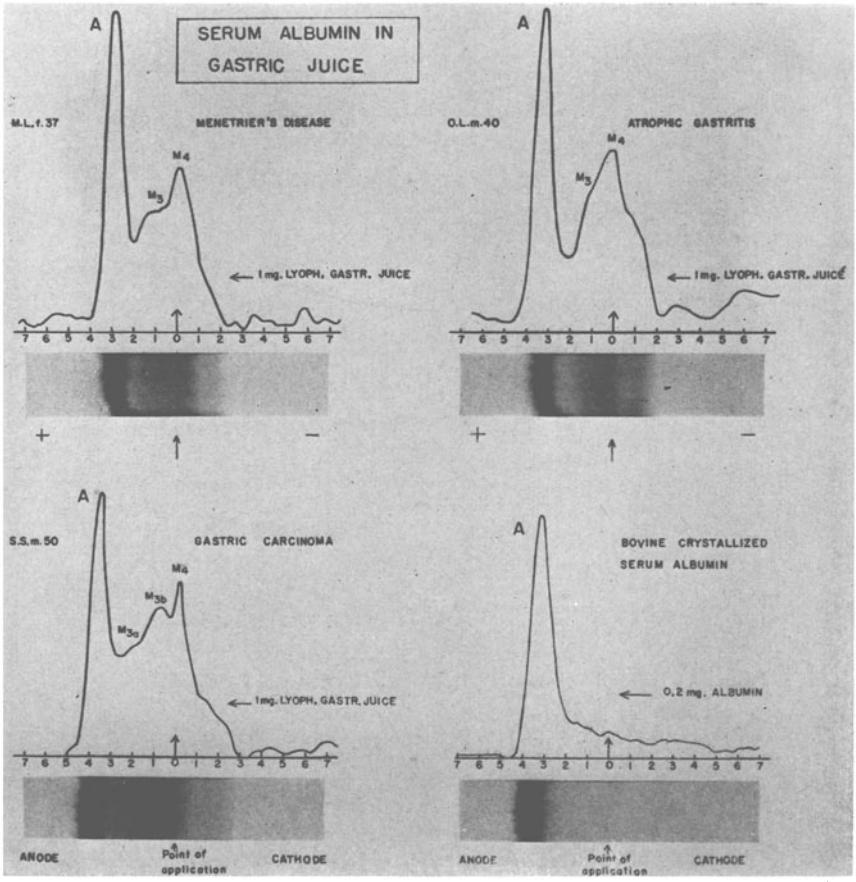
Fig. 17. Quantitation of serum albumin in 7 anacid gastric juices by paper electrophoresis. (Amido black stain)

globulin. This material, like gamma globulin (Fig. 19) was located slightly toward the cathode from the application point. It differed from the mucoprotein component M_4, however, by staining heavily with amido black 10B and SF light-green stains and by not staining

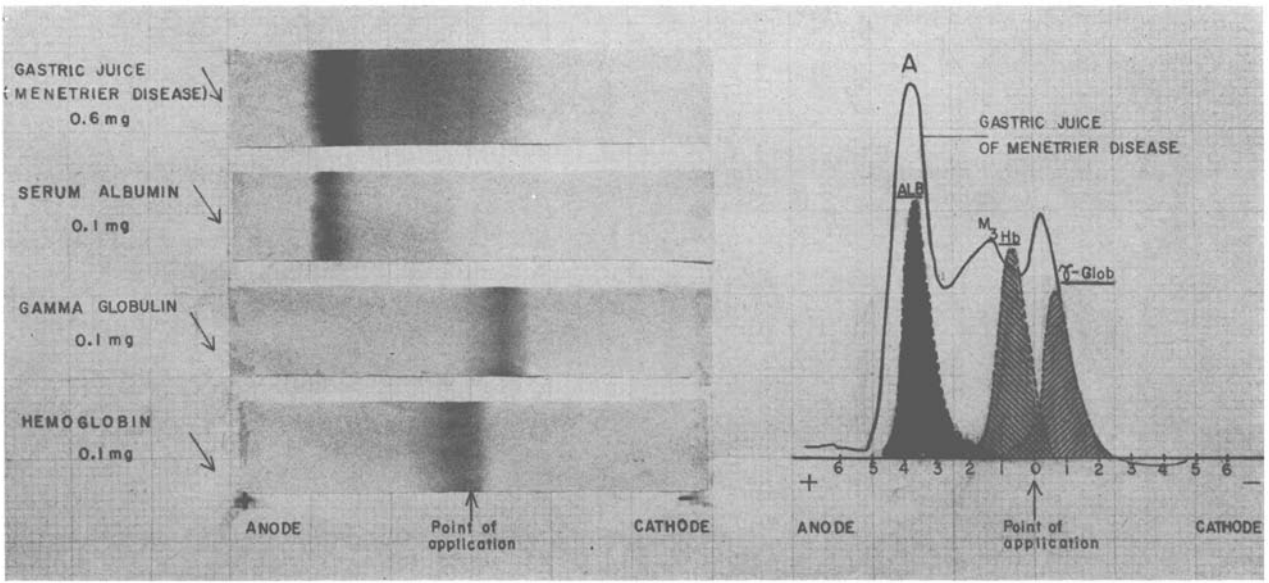


From *Current Gastroenterology*

Fig. 18. Massive albumin bands in electrophoretic tracings of patients with Menetrier's disease, atrophic gastritis, and gastric cancer. The tracing of bovine crystalline serum albumin is drawn for comparison. (Amido black stain)

with PAS stain. In these cases, not only serum albumin, but gamma globulin as well, had obviously passed from the blood into the gastric juice.

We were surprised to find this gamma globulin-like material in several anacid gastric juices where no serum albumin could be detected. This may have been due, in some instances, to contamination with bile. In several juices, however, which were collected from patients with *histamine-fast anacidity and advanced atrophic lesions of the gastric* mucosa, this globulin-like material was found where no bile was present. Immunoelectrophoretic work done in our laboratory by Ōhara has confirmed the gamma globulin nature of this material.[21]



From *Current Gastroenterology*

Fig. 19. Paper electrophoretic pattern of hemoglobin, serum albumin, gamma globulin, and gastric juice in Menetrier's disease. (Amido black stain)

DISCUSSION

Sufficient evidence is now at hand to warrant the conclusion that human gastric juice normally contains a certain amount of serum albumin. This is indicated by recent paper electrophoretic work done by Gullberg and Olhagen,[23] which has revealed a serum albumin band in normal acid gastric juice neutralized in vivo by the introduction of a phosphate buffer into the stomach before collection of the gastric secretion. Holman *et al.* and Hollander and Horowitz have found an albumin-like material with all the precipitation and mobility characteristics of serum albumin in normal human gastric juice[8] and in normal canine acetylcholine-stimulated gastric secretion.[24] That this component was serum albumin was confirmed by immunoelectrophoresis[24] and by use of the Ouchterlony immunological technic.[8] Armstrong *et al.*,[25] working with rats, also concluded that some serum albumin normally passes into the gastrointestinal tract and there undergoes physiological degradation, and that the G.I. tract participates in the normal metabolic turnover of serum proteins.

Our data indicate that the concentration of albumin in the anacid fasting gastric juice of individuals with no gastric pathology is below 10 mg./100 ml. of juice—i.e., below 0.01%. This value is low as compared with the concentration of albumin in the serum, and the amount of albumin present in the gastric juice in Menetrier's disease or gastric cancer. One must, therefore, make a sharp distinction between the normal passage of small amounts of serum albumin (and other serum proteins including gamma globulin) into the stomach, and the massive leakage of serum albumin into the gastric lumen in gastric cancer, Menetrier's disease, and some cases of advanced gastric atrophy.

The passage of small amounts of serum albumin into the stomach under normal conditions is in all likelihood due only to transudation. The mechanics of the excessive passage of serum proteins into the gastric juice, however may be very complex.

Several mechanisms may be instrumental in causing a massive protein leakage: (1) bleeding into the stomach; (2) exudation of serum from ulcerated surfaces of the gastric mucosa, as in gastric ulcer or cancer; (3) inflammatory exudation of serum from the gastric mucosa, as in gastritis; (4) excessive transudation of serum proteins, mainly albumin, as a result of increased vascularization, vascular permeability, and thinning of the mucosa; and (5) leakage of lymph into the gastric lumen from obstructed, dilated or eroded lymphatics.

Gastric Bleeding

Gastric bleeding lies outside the area of this discussion, since the passage of serum albumin and gamma globulin into the gastric juice

was not due, in most of our patients, to an admixture of blood. Evidence for this was (1) the absence of any reddish or brownish tinge to the gastric juice, and (2) the absence of a prominent hemoglobin peak in the electrophoretic tracings.* This indicated that the albumin or globulin leakage into the gastric juice was due, not to an admixture of blood, but rather to a leakage of serum from the blood into the gastric lumen.

The loss of albumin by bleeding, however, may be combined with serum leakage such as we have seen in a few patients with gastric cancer. In these cases, however, the ratio of the concentrations of albumin to hemoglobin was much higher than that in the blood where it normally is 1:5–7, or, in patients with severe blood loss, 1:3–4.

Exudation of Serum

Exudation of serum into the gastric lumen from ulcerated surfaces of cancerous gastric lesions was detected by the relatively crude technics of the time early in the present century and was reported by Solomon[26] and Wolff and Junghans.[27] Whether this mechanism is adequate to explain the massive leakage of serum albumin into the gastric lumen in all patients with cancer of the stomach remains to be seen.

Exudation of serum from the surrounding inflamed, and often atrophic, gastric mucosa may be an additional pathogenic factor. This may be similar to the process reported by Katsch 25 yr. ago in cases of what he termed "gastritis serosa."[28] More recently, this exudation of serum proteins from the inflamed gastric mucosa was recognized, also, by Norpoth *et al.*,[29] who correlated the ratio of total proteins in the gastric juice, as determined by biuret reaction, with the amount of protein contained in the gastric mucous fractions. In cases of gastritis, they found the total proteins to be in excess of that contained in the mucous fractions.

Transudation of Serum

Excessive transudation of serum into the gastric lumen in patients with gastric atrophy is probably also responsible, at least in part, for the passage of serum albumin into the stomach. This was first noted by Henning *et al.*[30] and was also reported by us,[11] in patients with pernicious anemia and advanced atrophic gastritis as confirmed by suction biopsy. In most instances, the presence here of gamma globulin was also detected. This would seem to point to the coexistence of an increased permeability due to atrophy, which may be associated with that of an inflammatory exudation due to gastritis.

*Hemoglobin, on electrophoresis, is located at the anodic side of the partition, close to the application point (Fig. 19).

Passage of Serum Albumin from Lymphatics

Passage of serum albumin from dilated, obstructed, or disrupted lymphatics and lymphatic lakes in the gastric wall was found to be responsible for some protein-losing enteropathies associated with hypalbuminemia, especially in the series run by Holman *et al.*[8] and by Schwartz *et al.*[2, 3] In their patients, a histological abnormality was found in the lymphatics in the wall of the small intestine. It is not yet known, however, whether the same mechanism is operative in Menetrier's disease. Here, a frequent edema within the lamina propria[31] may be indicative of some disturbance in the lymph flow. The congestion of the abundant vasculature in the submucosa,[31] a frequent finding in this disease, together with a thinning of the mucosa, associated atrophic lesions of the gastric glands, and the coexistence of inflammatory mucosal lesions,[31] may contribute to the excessive transudation and exudation of serum proteins into the gastric lumen.

In the interpretation of the presence of massive cathodic bands in the gastric electrophoregrams after steroid treatment,[22] two possible mechanisms may be considered: (1) Steroid treatment may cause increased albumin leakage into the gastric juice. The albumin would then undergo degradation by proteolytic enzymes in the acid gastric juice. This, in turn, would cause the formation of the massive cathodic bands. (2) The administration of steroids would cause the gastric mucosa to increase its secretion of polypeptides, which have cathodic mobilities similar to those of materials X_2, Y_1, Y_2. The situations may, of course, coexist.

The consistent incidence of massive leakage of serum albumin into the gastric juice in the majority of our patients with gastric cancer associated with anacidity assigns an important diagnostic role, we believe, to gastric paper electrophoresis in screening for cancer of the stomach. In the future it is possible that similar findings will be obtained in gastric cancer patients who secrete free acid and pepsin, and in whom the peptic digestion of serum albumin will be prevented by the intragastric instillation of neutral buffers prior to, and during intubation.[23]

SUMMARY

Serum albumin was found in 40 anacid gastric juices of more than 400 gastric paper electrophoregrams done on over 300 persons with and without gastric disease. When the concentration of albumin was high, the albumin showed as a prominent and unusually heavily amido-black-stained band in the anodic area of the partition. This material had

the electrophoretic mobility of serum albumin and was precipitable with trichloroacetic acid, the precipitate being soluble in 95% ethanol.

In acid- and pepsin-containing gastric juices collected without intragastric neutralization, the presence of the degradation products of serum albumin was recognized on the cathodic side of the partition. In patients with duodenal ulcer or gastric hypersecretion, these degradation products were usually present at high concentration.

In half the 40 anacid gastric juices that showed the presence of a serum albumin band, the concentration of serum albumin in the gastric juice was relatively low (below 20 mg./100 ml.). In these cases, with the exception of 2 cases of gastric malignancy, it ranged from 3 to 14% w/w of the nondialyzable solids. Eight of these anacid juices were from patients whose gastric secretion was anacid under fasting conditions but contained free hydrochloric acid and pepsin after stimulation. Here the concentration of albumin did not exceed 8% w/w of the nondialyzable solids of gastric juice and was below 10 mg./100 ml. gastric juice.

In 19 instances, we found a massive leakage of serum albumin into the gastric juice with a content of serum albumin ranging from 15 to 63% w/w of nondialyzable solids of gastric juice and from 20.0 to 482.0 mg./100 ml. of native juice. These instances of massive albumin leakage included 10 of our 12 patients with gastric cancer with histamine-fast anacidity, 1 with pernicious anemia, 1 with sprue, 5 with Menetrier's disease associated with hypalbuminemia, and 2 with steroid-treated rheumatoid arthritis.

In some anacid gastric juices, an additional component was found that had the electrophoretic mobility of gamma globulin and migrated slightly by endosmosis toward the cathode. This material represents gamma globulin which passed, usually with serum albumin but sometimes without it, from the blood into the gastric lumen.

CONCLUSIONS

The presence of serum albumin in the gastric lumen can be readily diagnosed by paper electrophoresis of the gastric juice. While small amounts of albumin are present in the gastric juice under normal conditions, a massive leakage of serum albumin into the stomach occurs often in gastric cancer, Menetrier's disease, and in some cases of gastric atrophy. Paper electrophoresis of the gastric juice has definite clinical significance for the detection of these conditions.

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