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THE EFFECT OF AN ANION EXCHANGE RESIN ON PEPSIN

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INTRODUCTION

The importance of pepsin in the pathogenesis of peptic ulcer

The causes of peptic ulcer are still unknown. Two of the main factors are, according to Ivy, Grossman & Bachrach, 1950:

a) The mucous membrane factor, causing a locus minoris resistentiae.

b) The pepsin-HCl-factor which may develop autolysis. There are two chemical factors in the gastric juice of importance for autolysis:

1) HCl, which may act directly on the mucous membrane or indirectly via its pepsin activating ability.

2) Proteolytic enzymes. Rennin probably is not present in adults (Cantarow & Trumper, 1949). Kathepsin has its optimum at pH 4—5 (Evans, 1945), and probably is of no importance. There remains only pepsin with its optimum at pH 1—2 (Northrop, Kunitz & Herriott, 1948).

Several experimental data indicate the importance of pepsin in the pathogenesis of peptic ulcer. Thus LeVeen (1947) maintains that HCl has only an indirect effect. A weak solution of HCl with a high pepsin activity produced more frequent and larger ulcers than a strong solution with a low pepsin activity. Any therapy which reduces the peptic activity should be of interest.

Ion exchange resins

Antacida usually have no other effect than that of neutralizing the acid (Warren, Front & Kirsner, 1943). With the anion exchange resins a new principle has been introduced in the therapy of peptic ulcers. The use of ion exchange resins was suggested in 1943 by Skogseid (1951), and introduced by Segal, Hodge, Watson & Scott in 1945. Segal *et al.* used Amberlite IR-4. They maintain that this resin has no direct effect on pepsin, but has an indirect inactivating effect by increasing the pH.

In a later publication it is demonstrated that Amberlite IR-4 inactivates 80 per cent of the pepsin at pH 1 (n/10 HCl). Great quantities of the resin were used (3 g to 100 mg of pepsin). Trypsin was inactivated in the same manner, and this inactivation was irreversible, but it is not mentioned whether this also applies to pepsin (Martin & Wilkinson, 1946).

At Rikshospitalets Medisinske Poliklinikk, Oslo, good results have been obtained with an ion exchange resin in the treatment of peptic ulcer (Dedichen, 1952).

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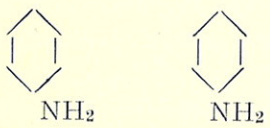
[1] With the technical assistance of Miss M. Pedersen.

The object of the present investigation has been to study the mode of action of the anion exchange resin on pepsin-HCl solutions and on gastric juice in vitro. It has been attempted to answer the following questions: 1. Is the pepsin affected by the anion exchange resin? 2. To what degree? 3. In which way?

The general properties of the anion exchange resin

The anion exchange resin used in this study was polyaminostyrene:



(It is manufactured by Norsk Hydro-Elektrisk Kvælstofaktieselskab (Skogseid, 1948, 1951), and is compounded and distributed by Nyegaard & Co A/S under the trade name Macrin "Nyco".)

The brownish material is insoluble in all the usual solvents. It is an anion exchange resin, a weak base which give salts with strong acids. The salts are also insoluble in water.

The experiments have been carried out with a commercial preparation from the same batch without further purification. It has been screened according to the standards of Ph. N. 5th Ed. 1939.

METHODS

For the pepsin determinations Hunt's method has been used with dried, human plasma as substrate (Hunt, 1948). The readings were made in a Spekker photoelectric absorption meter (Hilger) and for some analyses in a Beckman spectrophotometer. All analyses have been made in duplicate with a few exceptions which are mentioned in the text. Hunt's standard is used, and the pepsin activity is given in Pepsin Units (P. U.) according to Hunt's definition.

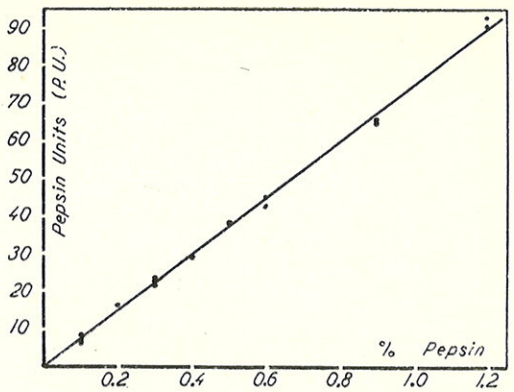


Fig. 1. Accuracy of the estimation of pepsin by Hunt's method.

Hunt states the error to be ± 2.64 per cent, and Linde has found ± 4 P. U./ml (Linde, 1950). Such calculations of error are not made in this work, but the diagram in Fig. 1 is an expression of the usefulness of the method (no measurements are omitted).

The pH determinations were made with a glass electrode in a Cambridge portable pH-meter. The error of measurement is ± 0.05.

For the experiments with pepsin, Pepsinum concentratum (Langebeck) 1 : 2000 was used. (For the greater part of the experiment B. 4. a different and apparently stronger portion was used.) Filtration reduces the pepsin activity, all analyses were therefore filtered in the same way.

The stirring was done in a glass beaker with an electric agitator.

The diagrams illustrate the results of representative single experiments, and are not constructed as statistical average curves.

EXPERIMENTS

A. *The acid binding capacity of Macrin*
Procedure: Macrin of 30 mesh in quantities from 0 to 5 per cent was added to

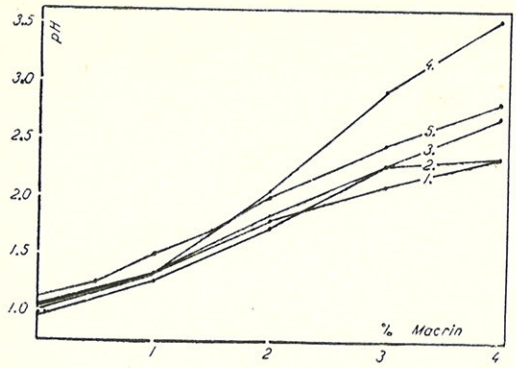


Fig. 2. The effect of Macrin on the pH.
1. HCl.
2. » with 1 mEq NaCl per L.
3. » » 10 » »
4. » » 100 » »
5. » » 1 per cent of pepsin.

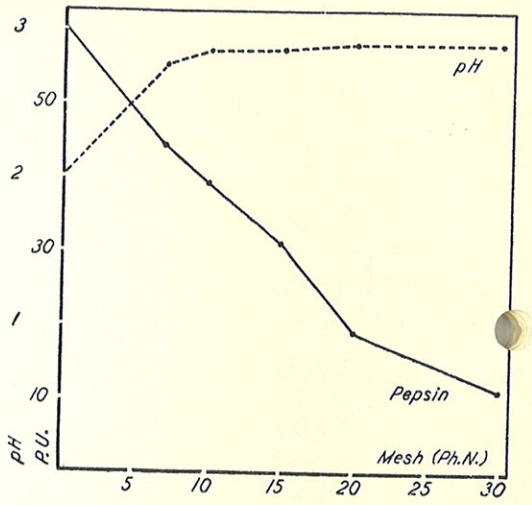


Fig. 3. The grain size of Macrin.

approximately n/10 HCl. It was stirred for 20 minutes, filtered, and pH was determined in the filtrate. The same test was made with the addition of 1 per cent pepsin and varying quantities of NaCl.

Results: See Fig. 2.

Comments: The increase in pH shows an approximately linear relationship to the quantity of Macrin (in the range which was investigated). With the addition of pepsin and NaCl the increase in pH was more marked.

B. *The pepsin-inactivating effect of Macrin (Pepsin-HCl solutions)*

1. *The importance of the grain size*

Procedure: A solution of 1 per cent pepsin in HCl was adjusted to pH 2.1. Macrin of decreasing grain sizes, in a quantity of 1 per cent weight, was added to aliquots. After stirring for 20 minutes and filtering, pH and pepsin were determined in the filtrate.

Results: See Fig. 3.

Comments: The inactivation of pepsin increases when the total surface of the Macrin is enlarged. The same holds true for the acid binding capacity. This is in accordance with the observations of Martin & Wilkinson (1946). The relation is different for pepsin and for pH. The inactivation of pepsin is approximately inversely proportional to the grain size, whilst pH does not increase further when the grain size is reduced below a certain lower limit.

2. *The importance of the stirring time*

Procedure: The same pepsin-HCl solution was used as in the previous experiment, and Macrin of 30 mesh was added in a quantity of 1 per cent. Aliquots were stirred from 20 to 1 minutes, filtered immediately, and pepsin and pH were determined in the filtrate. The experiments were repeated with a 1.5 per cent solution of pepsin with 1.5 per cent Macrin added. Test were also made without the addition of Macrin.

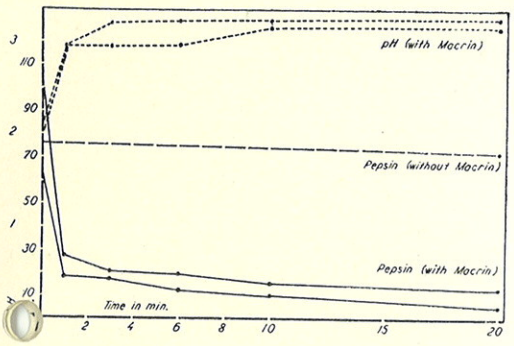


Fig. 4. The time of stirring.

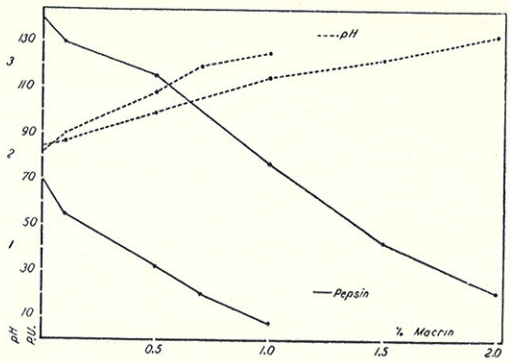


Fig. 5. The quantity of Macrin.

Results: See Fig. 4.

Comments: The inactivation of pepsin increases with the stirring time, but after the first minute the increase is very slight. The pH reaches its maximum during the first minute.

3. *The importance of the quantity of Macrin*

Procedure: 1 and 2 per cent solutions of pepsin in HCl were adjusted to pH 2.1. Macrin of 30 mesh was added to aliquots in quantities varying from 0 to 2 per cent by weight. The mixtures were stirred for 20 minutes, filtered, and pH and pepsin were determined in the filtrates.

Results: See Fig. 5.

Comments: The inactivation of pepsin under these circumstances is approximately proportional to the quantity of Macrin.

4. *How does Macrin inactivate the pepsin?*

Macrin may only have an indirect effect on the pepsin, caused by the increase in the pH, or it may act directly on the pepsin. To answer this question, the relation between the pepsin activity and the pH was first investigated.

Procedure: 1 per cent pepsin solutions, with pH ranging from 1 to 4, were made, kept in refrigerator over night, and then the pepsin was determined. The pH of the substrate was adjusted in accordance with the pepsin solutions.[1] — The experiment was repeated, the pH of the substrate being constant at 2.1, the pH of the pepsin solutions ranging from 1 to 7. These tests were not made in duplicate.

Results: See Fig. 6.

Comments: The experiment with adjusted substrate shows the total reduction in peptic activity as the pH increases. This reduction is roughly proportional to the increase in the pH in the interval from pH 2 to pH 4. — The experiment with constant substrate shows the irreversible part of the inactivation. In the interval from pH 2 to pH 4 the inactivation is mainly reversible.

The pepsin inactivation of Macrin may therefore be ascribed to the increase of the

[1] The adjustment of the pH in the substrate in this and the following experiments is quite essential. It was suggested by the Research Division of the Glaxo Laboratories in England.

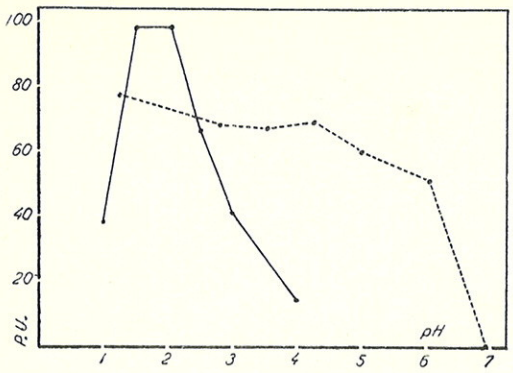


Fig. 6. The activity of pepsin at different pH.
— — — — pH of the substrate is constant.
———— pH of the substrate is adjusted.

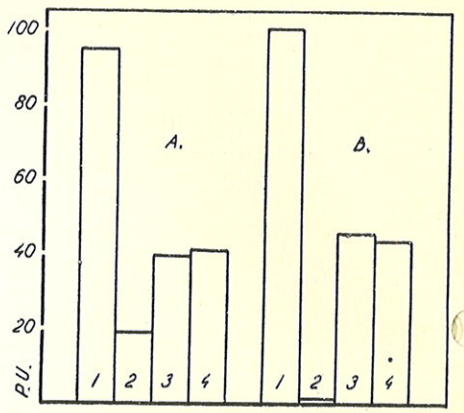


Fig. 7. The inactivation of pepsin with Macrin and with NaOH.

		pH A	pH B
1.	Pepsin-HCl	2.03	1.52
2.	» and Macrin. Filtered....	3.00	2.80
3.	» and Macrin. Not filtered	3.00	2.80
4.	» and NaOH	3.00	2.80
	Macrin added	1 %	2 %

pH. To settle this question, the effect of Macrin is compared to that of NaOH.

Procedure: A 1 per cent solution of pepsin in HCl was adjusted to pH 2. One portion (1) was filtered, pH and pepsin being determined in the filtrate. To another portion (2) 1 per cent of Macrin was added, the mixture stirred for 10 minutes, filtered, and pH and pepsin determined in the filtrate. The third portion (3) was filtered, 1 per cent of Macrin added, the mixture stirred for 10 minutes, and pH and pepsin determined without the Macrin being filtered off. The fourth portion (4) was adjusted to the pH of (2) and (3) with NaOH. The substrates had the same pH as the samples. The experiment was repeated, starting at pH 1.5, 2 per cent of Macrin being added.

Results: See Fig. 7.

Comments: Macrin clearly has a double effect:

1) One part of the pepsin inactivation is due to the increase of the pH. This effect is identical with that of alkali, and if the Macrin is not removed, this one is the sole effect.

2) The other part is due to the absorption of pepsin. The Macrin being filtered off, this effect is in part added to the first one. If it is not removed, the absorbed pepsin appears to have the same peptic activity as free pepsin. The pepsin absorbed to Amberlite IR-4 also shows considerable peptic activity (Wilkinson & Martin, 1946). At the same pH the effect of absorption becomes greater as increasing quantities of Macrin are added.

5. *The influence of the pH on the pepsin absorption of Macrin*

Procedure: Two 1 per cent stock solutions of pepsin were made, the pH adjusted to 1 and 2. To aliquots of each solution, in-

creasing quantities of 30 mesh Macrin were added. Each sample was stirred for 20 minutes, filtered, and pH and pepsin determined in the filtrate. These tests were not made in duplicate.

Results: See Fig. 8.

Comments: In the solution originally adjusted to pH 2, the pepsin activity decreased quickly about proportionally to the increas- quantities of Macrin (see experiment B. 3). In the solution starting at pH 1, however, the pepsin activity did not decrease markedly until the pH had been increased above 2. Then the curve is about parallel to the one of the other solution.

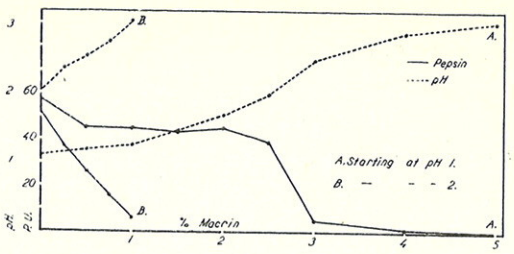


Fig. 8. The influence of pH on the pepsin absorption by Macrin.

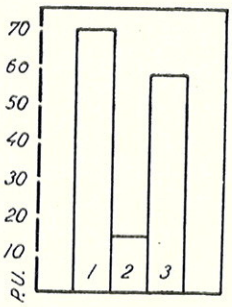


Fig. 9. The reversibility of pepsin absorption by Macrin.

1. Pepsin-HCl (pH 2.10).
2. » and Macrin (pH 3.06).
3. » and Macrin. HCl added before filtering (pH 2.00).

6. *The reversibility of the pepsin absorption of Macrin*

Procedure: A 1 per cent pepsin solution was adjusted to pH 2.1. One portion (1) was filtered, and in the filtrate pH and pepsin were determined. To the rest, 1 per cent of 30 mesh Macrin was added, and the mixture stirred for 20 minutes. One half of this portion (2) was filtered, pH and pepsin being determined in the filtrate. The other f (3) was brought down to pH 2.1 with a few drops of 2 n HCl, after shaking settled for 10 minutes at room temperature, filtered, and pH and pepsin were determined in the filtrate.

Results: See Fig. 9.

Comments: In this pH interval the greater part of the pepsin could be reactivated with the addition of HCl.

The pepsin was reactivated by lowering the pH to the original value, approximately 2. Experiment B. 5. suggested that the pepsin could not be reactivated until the pH had come down into this region, even if the original solution had a higher pH. The following experiment should corroborate this statement:

Procedure: A 1 per cent pepsin solution in HCl was adjusted to pH about 3. One portion (1) was filtered, and pH and pepsin were determined in the filtrate. To the rest was added 1 per cent of 30 mesh Macrin which was stirred for 20 minutes. A small portion (2) was then filtered, and pH and pepsin were determined in the filtrate. The remainder was divided into four portions

(3 a, b, c, d). Each portion was brought down to the pH wanted, after shaking settled for 10 minutes, filtered, and pH and pepsin were determined in the filtrate.

Results: *See* Fig. 10.

Comments: Pepsin was not reactivated when the pH was lowered to the original value of about 3. The reactivation did not occur until the pH had come down to about 2.

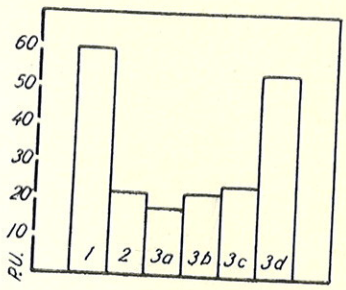


Fig. 10. The reversibility of pepsin absorption by Macrin.

1. Pepsin-HCl (pH 2.80).
2. » and Macrin (pH 3.60).
3. » and Macrin. Increasing amounts of HCl added before filtering:
   a) pH 3.22
   b) » 2.88
   c) » 2.52
   d) » 2.00

C. *The effect of Macrin saturated with HCl*

These experiments should indicate whether the effect of Macrin changes when the active amino group is combined with HCl. The experiments were made with a Macrin preparation which had been saturated with HCl. (The usual commodity had been steeped in concentrated HCl, dried and sieved through a 30 mesh screen.)

Procedure: A 1 per cent pepsin solution in HCl was adjusted to pH 2.1. 1 per cent by weight of the preparation was added, the mixture stirred for one hour, filtered, and pH and pepsin were determined in the filtrate. Check tests were made without any admixture.

Results: See Fig. 11. The results of experiment B. 2 are included for comparison.

Comments: pH and pepsin both showed a small, but constant fall. The fall in pepsin activity was considerably less than that obtained with the usual Macrin. The effect could possibly be explained by the diffusion of HCl from Macrin-HCl out into the solution, the pepsin taking the vacant places. The pepsin inactivation roughly corresponds to the expected, if the fall in pH is taken as an expression for the liberated Macrin (see experiment B. 3.) The results show that the active amino groups are necessary for the inactivation of pepsin.

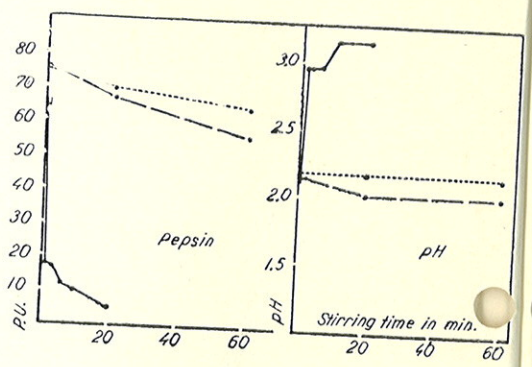


Fig. 11. The effect of Macrin, saturated with HCl, compared to the effect of Macrin.
····· Pepsin-HCl.
——— » and Macrin.
— — — » and Macrin, saturated with HCl.

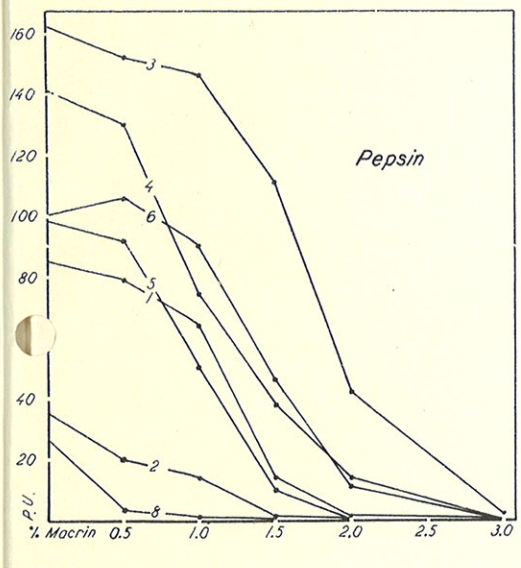


Fig. 12. The effect of Macrin on gastric juice.

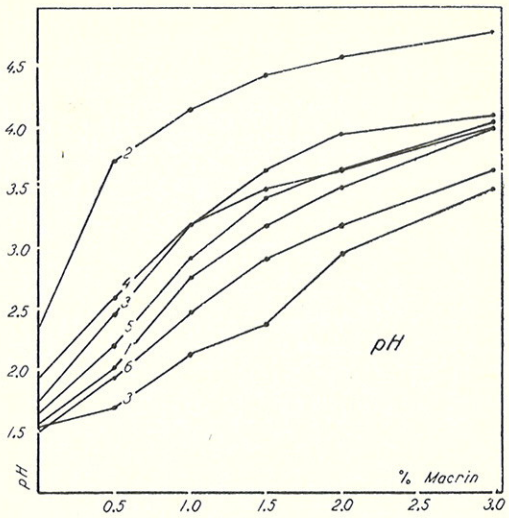


Fig. 13. The effect of Macrin on gastric juice.

D. *The effect of Macrin on gastric juice*

Procedure: The investigations were carried out in the gastric juices of eight consecutive patients, on whom gastric aspirations were performed for diagnostic reasons. The aspirations were performed in the morning, on an empty stomach. If less than 40 ml were obtained, 2 ml 1/4 ‰ Histamine were given subcutaneously and the aspiration continued (see Table 1). The gastric juice was filtered, and pH determined in the filtrate. Then equal portions of HCl with pH 2.1 were added (cf. Hunt, 1948). Increasing quantities of 30 mesh Macrin were added to aliquots, the mixture stirred for 20 minutes, filtered, and pH and pepsin were determined in the filtrate. The pH in the substrate was constant at pH 2.1.

Results: See Figs. 12 and 13. The numbers on the curves correspond to the numbers in the table.

Comments: The pH increased fairly uniformly, but did usually not exceed 5. Three per cent by weight of Macrin reduced the acidity to about 1/100 of its original value. The pepsin activity decreased. In the beginning the inactivation was small, but increased vigourously when the quantity of Macrin reached above a certain level, differing for each sample. Above this level, the pepsin activity fell evenly. The very weak gastric juices inactivated more slowly. The bend in the curves towards the end is probably artificial, caused by the fact that the pepsin determinations have not been made at adequately small intervals.

DISCUSSION

1. *The effect on the pH*

Macrin increases the pH in an acid solution. The acid binding capacity is approximately 6 mEq/g at pH below 2.

These experiments show that the pH continues to increase, in any case to 4, if the quantity of Macrin is adequately increased. The addition of pepsin and NaCl causes the pH to increase more quickly. In vitro the pH can be increased above the usual upper level in the stomach, but for this very high dosages of Macrin are needed, probably far above the usual dosage. (3 per cent of Macrin did, for instance, increase the pH from 1.5 to 3.5 in a gastric juice.) It is not known whether this is followed by a rebound in gastric acidity (Wirts & Rehfuss, 1950).

2. *The effect on pepsin*

Macrin inactivates pepsin, partly by increasing the pH, partly by absorption.

The effect of increasing the pH is studied in experiment B. 4. It starts at pH 2, and then proceeds about proportionally to the increase in the pH. It is identical with the effect of alkali, and is dependent only on the pH, not directly on the concentration of Macrin. It is mainly reversible. When the Macrin is not filtered off, this effect will be the only one.

The effect of absorption is studied separately in the experiments with a constant pH in the substrate. The effect of increasing the pH does not come into play under these experimental conditions.

Macrin seems to start its absorption of pepsin when the pH is increased to about 2, because:

1) In a pepsin-HCl solution the pepsin activity does not decrease markedly before the pH is increased to about 2 (experiment B. 5). The same seems to hold for the gastric juices (experiment D).

2) The pepsin can be reactivated from the Macrin-pepsin complex if the pH is lowered to approximately 2, whilst no reactivation takes place above this level (experiment B. 6).

Skogseid has shown that: "By increasing the pH (say above 2) an increasing number of the Macrin's amino groups will appear in a free state" (Skogeid, 1951). Therefore, the absorption of pepsin probably presupposes free amino groups. Note that Macrin which was saturated with HCl had no effect (experiment C). The effect is therefore no sheer physical absorption.

HCl probably has the first priority on the Macrin. Above pH 2, the pepsin can dispose of the free amino groups, and the absorption then proceeds proportionally to the quantity of Macrin. The absorption amounts to approximately 60—70 P. U. for each per cent of Macrin (experiment B. 3). In the gastric juices the absorption is of the same order, when the pH is above 2 (experiment D).

The effect of absorption may also be studied as the difference between the total effect and the effect caused by the increasing of the pH (experiment B. 4). In the experiments this effect appears less than expected according to experiment B. 3, the reason being as yet unrevealed. Above pH 2 it is not dependent on the pH, but on the concentration of Macrin. Absorbed pepsin apparently has the same effect as free pepsin. The effect of absorption may therefore only be demonstrated when the Macrin is filtered off.

The absorption of pepsin is reversible (experiment B. 6). No essential change can, therefore, take place in the pepsin molecule

during the absorption. This corresponds with the fact that the Macrin-pepsin complex seems to have the same peptic activity as free pepsin (experiment B. 4).

The inactivation of pepsin is firstly dependent on the pH and the dosage of Macrin, secondly it is dependent on the Macrin being filtered off. When the Macrin is not filtered off, the effect is that of alkali. On filtering off, the effect becomes greater (provided the removal takes place before the pH is decreased to such a degree that the pepsin is eluted). It is a matter of opinion whether the calculation of dosage in vivo should be based on the supposition that Macrin is removed. Sooner or later the Macrin is carried out of the stomach. Therefore it seems justified to base the calculation on the supposition that Macrin, at least partly, is removed. In spite of this, the theoretical dosage necessarily has to be fairly high, far above the usual clinical dosage, but such an estimate will always be encumbered with a great factor of uncertainty, and will therefore be of minor value. In practice the effective dosage varies within wide limits. With Amberlite IR-4 the dosage varied between 0.5 and 102 g per 24 hours, with an average of 24 g (Spears & Pfeiffer, 1947). The point is that Macrin can inactivate the gastric juice, and the supplied quantity may be adequate. This has to be determined by clinical experience, and the dosage can only be fixed empirically.

Table I. *Survey of the gastric juices which have been investigated*

No.	Age	Sex	Diagnosis	Histamine given	pH in pure gastric juice	P.U.
1	29	♂	Obstipatio spastica	+	1.35	85
2	32	♂	Gastritis	+	2.59	35
3	35	♂	Ulcus duod.	+	1.22	162
4	43	♀	Anemia sideropenica	—	1.70	141
5	21	♂	Gastritis	—	1.44	98
6	43	♂	Dyspepsia	+	1.22	100
7	19	♂	Obstipatio spastica	—	2.86	1
8	57	♀	Dyspepsia	+	1.62	26

CONCLUSIONS AND SUMMARY

1. Polyaminostyrene (Macrin) increases the pH in a hydrochloric acid solution approximately proportionally to the quantity of Macrin. This has been investigated for the pH range from 1 to 4.

2. Macrin can inactivate pepsin:

a) The effect increases with decreasing grain size. The mode of action is here somewhat different for the pH and for pepsin.

b) The effect takes place quickly, mainly within a minute.

c) The effect does not start before the pH is above about 2.

d) The effect is a double one. Firstly Macrin reduces the peptic activity by increasing the pH, secondly by absorbing pepsin. When the Macrin is filtered off, both effects can be demonstrated. If it is not removed, the effect is identical with that of

alkali. The absorbed pepsin therefore apparently has the same activity as free pepsin.

e) The effect is mostly reversible.

f) Macrin which has been saturated with HCl is practically inactive, thus it can not be assumed that Macrin acts by physical absorption.

3. In gastric juice Macrin increases the pH and absorbs pepsin in the same manner as in the pepsin-HCl solutions. Its clinical use in peptic ulcer seems therefore theoretically well founded. The question of dosage has to be settled by clinical trial.

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