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THE REGULATION OF GASTRIC EMPTYING OF MEALS CONTAINING CITRIC ACID AND SALTS OF CITRIC ACID

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It is known that when test meals containing hydrochloric acid are given, the greater the concentration of acid in the meal the slower is gastric emptying (Shay & Gershon-Cohen, 1934; Van Liere & Sleeth, 1940; Pathak, 1959; Hunt & Pathak, 1960). This slowing of gastric emptying is believed to depend upon the stimulation of duodenal receptors by the duodenal contents recently transferred from the stomach. The present studies are concerned with the rates of emptying of test meals containing citric acid and the various sodium salts of this trivalent acid. These were chosen for study because they allow the preparation of solutions with a variety of pH values and with various degrees of buffering power which can be used to decide on the relative importance of these two variables in exciting the receptors concerned.

METHODS

The subjects were four volunteer students and HT. Test meals were given down a tube at 37° C to the fasting subjects after their stomachs had been washed out with 250 ml. of tap water, which was recovered as completely as possible before the meal was given. All meals contained about 60 mg phenol red/l. to act as a marker. Phenol red is not absorbed or secreted by the stomach (for references see Hunt, 1947). After an interval, constant for each subject but varying between 9 and 19 min, the meal was withdrawn through the tube; when this recovery appeared to be complete 250 ml. tap water was given and this portion was aspirated separately. The amount of phenol red in this second aspirate allowed the volume recorded for the first recovery to be corrected for incompleteness. When a recovery began 9 min after giving the meal, the meal was recorded as of 10 min duration to allow for the time required to recover the gastric contents. Other times were similarly adjusted by 1 min.

The majority of the test meals were made up from appropriate amounts of citric acid and sodium citrate. Test meals containing ammonium citrate or ammonium chloride were made up with the corresponding salts.

Estimation of phenol red. Ten millilitres of the test-meal solution or of the recovered gastric contents and 50 ml. of a solution of Na_3PO_4 (27·5 g/l.) were pipetted into a 250 ml. flask which was then filled up to the mark with distilled water. The mixture had a pH of about 11–12. The optical density of the purple solution so produced was estimated in a single cell photo-electric colorimeter with a green filter (Ilford 404). The concentration of phenol red was then determined from a curve relating concentration of phenol red to optical density. The green filter used minimized the effect of bile.

Expression of results. In each instance the volume of the original meal recovered from the stomach was calculated from the expression

$$\text{Volume gastric contents} \times \frac{\text{concentration phenol red in gastric contents}}{\text{concentration phenol red in original meal}}.$$

For each subject and each solute a plot was made of volumes of meal remaining in the stomach after a fixed interval against the concentration of the solute in the given meal. A line drawn through these points was usually straight to the eye over part of the range of concentrations of each solute: for this part of the findings a straight line was fitted by the method of least squares. Data for the regression equations of these lines are given in Table 1. The lines relating the volume of meal remaining, on the ordinate, to concentration of citric acid and salts of citric acid over the ranges studied, are shown in Fig. 1. The curved parts of the lines were drawn by eye through the mean values for several observations. When the volume of the meal recovered was in excess of about 550 ml. further increase in concentration of solute did not always proportionately increase the volume of meal recovered.

TABLE 1. The effect of citric acid and salts of citric acid and ammonium chloride in a test meal on the volume of a 750 ml. meal remaining in the stomach after a fixed interval

	JS	JN	SW	BY	HT
Age	22	19	22	21	44
Weight (kg)	72	59	63	70	59
Height (cm)	175	168	180	175	175
Total no. of tests	170	100	138	113	151
Time (min)	10	10	15	15	20
Citric acid					
a	368	413	291	277	268
$b \pm$ S.E.	1·31 ± 0·16	1·45 ± 0·098	2·20 ± 0·55	2·79 ± 0·42	1·59 ± 0·25
R (no.)	10–200 (9)	10–150 (8)	10–150 (9)	25–120 (7)	10–200 (14)
Dihydrogen sodium citrate					
a	368	358	315	301	191
$b \pm$ S.E.	0·68 ± 0·19	1·28 ± 0·33	1·01 ± 0·13	1·15 ± 0·13	1·41 ± 0·14
R (no.)	45–300 (17)	30–150 (13)	15–300 (36)	30–300 (16)	30–270 (11)
Hydrogen disodium citrate					
a	322	179	124	248	66
$b \pm$ S.E.	0·24 ± 0·15	1·34 ± 0·18	1·17 ± 0·17	0·98 ± 0·15	1·01 ± 0·14
R (no.)	45–300 (23)	75–300 (20)	75–300 (20)	45–300 (23)	100–400 (23)
Trisodium citrate					
a	169	275	110	251	83
$b \pm$ S.E.	0·31 ± 0·12	0·25 ± 0·13	0·50 ± 0·07	0·23 ± 0·12	0·52 ± 0·12
R (no.)	100–500 (29)	100–500 (12)	100–500 (23)	100–500 (28)	100–500 (26)
Ammonium citrate					
a	354	376	—	—	262
$b \pm$ S.E.	0·36 ± 0·50	0·014 ± 0·036	—	—	1·22 ± 0·60
R (no.)	0–100 (14)	0–75 (15)	—	—	0–50 (15)
Ammonium chloride					
a	323	357	—	—	237
$b \pm$ S.E.	0·019 ± 0·29	0·047 ± 0·035	—	—	0·41 ± 0·45
R (no.)	0–70 (23)	0–90 (8)	—	—	0–100 (13)

Volume of meal remaining (y ml.) plotted against concentration of solute (x m-equiv./l.) $y = a + bx$, where a corresponds to the volume calculated to remain with zero concentration of solute, that is, the intercept of the regression line and the ordinate. R (no.) = Range of concentrations (number of experiments).

For this reason the calculated regression lines were extended at high concentrations to cover only the linear part of the relationship between concentration of solute and volume of meal recovered.

RESULTS

The data for the five subjects taking meals of citric acid and its sodium salts are shown in Fig. 1. The durations of the tests, which were constant for each subject, were adjusted to give about 300–400 ml. of recovered meal when it was pure water. This is of importance in between-subject comparisons of the effects of the solutes on gastric emptying. Had there been, say, only 100 ml. of test meal leaving the stomach with no solute in the meal it would have been difficult to assess quantitatively any decrease in the rate of emptying.

There are some features common to all five subjects:

(1) Initially as the concentration of trisodium citrate in the meal was raised the volume of meal remaining after a fixed interval fell until a concentration of between 50 and 100 millinormal (mN) was reached. Thereafter at higher concentrations of trisodium citrate the volume recovered increased.

(2) A similar pattern of response was seen with hydrogen disodium citrate, but the concentration of citrate ion giving minimal volume of meal recovered was less than with the trisodium salt.

(3) In general the increase in volume of meal recovered per increase in concentration of citrate ion was greatest for citric acid, followed by dihydrogen sodium citrate, hydrogen disodium citrate and trisodium citrate, in that order.

(4) For reasons which will appear later, some similar studies were made with ammonium citrate and ammonium chloride. It may be seen from Table 1 that over the range of concentrations studied, 50–100 mN, there was no significant increase in the volume of the meal recovered as the concentration of solute in the original meal was raised.

The results for the volume of meal remaining in HT with concentrations of hydrogen disodium citrate less than 100 mN present a special feature, namely a hump in the curve at about 50 mN. The mean volume remaining with 30 mN solutions was 171 ml. (S.E. $\pm$ 12, 7 observations), whilst at 45 mN the mean volume was 239 ml. (S.E. $\pm$ 16, 16 observations), the difference being 68 ml. (S.E. $\pm$ 20), which is statistically highly significant. When all the results for volume of meal remaining with concentrations between 60 and 100 mN hydrogen disodium citrate were plotted, the slope of the line was $-1{\cdot}59$ (S.E. $\pm 0{\cdot}22$; 22 observations). Taking these two statistical tests together, it is very unlikely that the hump in the curve for hydrogen disodium citrate for HT is a chance affair. None of the other irregularities in the curves in Fig. 1 was statistically significant in spite of many repetitions of the tests.

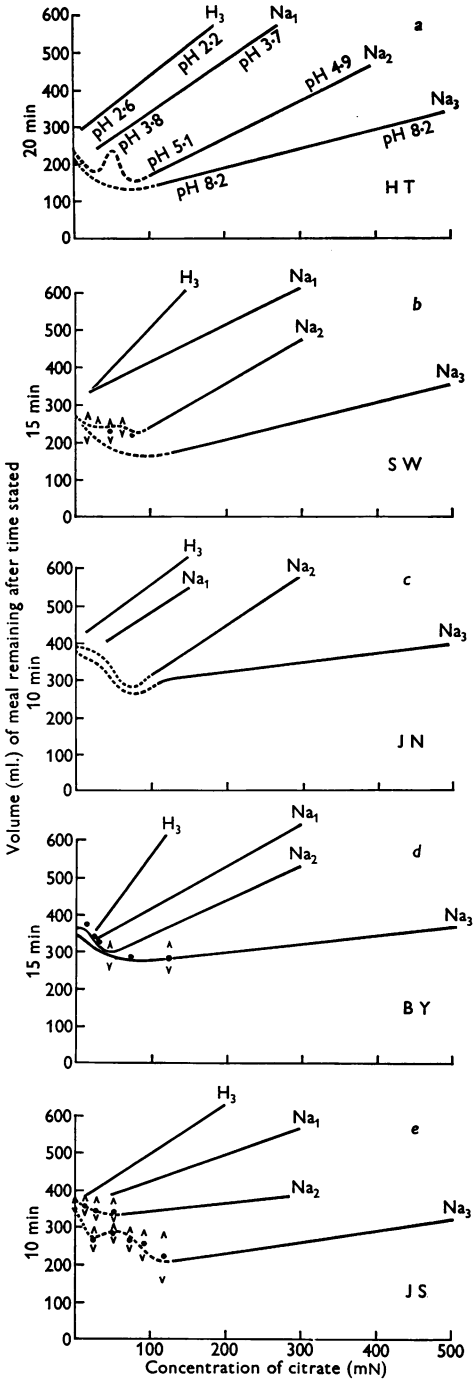

Fig. 1. Volumes of meal remaining in the stomach after a fixed interval plotted against concentration of solute in meal. H_3, citric acid; Na_1, dihydrogen monosodium citrate; Na_2, monohydrogen disodium citrate; Na_3, trisodium citrate. Ordinates, volume (ml.) of meal remaining at time stated; abscissae, concentration of solute (mN).

DISCUSSION

As a result of a previous study of gastric emptying with test meals containing various electrolyte and non-electrolyte solutes (Hunt & Pathak, 1960) it was suggested that gastric emptying was slowed by a duodenal osmoreceptor. The osmotic pressure of the duodenal contents, recently transferred from the stomach, was regarded as reducing the flux of water from the duodenum into a sensitive vesicle which, being thus affected, gave rise to a signal which slowed gastric emptying by reducing the vigour of gastric peristalsis. The response to the majority of solutes was a degree of slowing directly proportional to the concentration in the test meal, but with low concentrations of sodium chloride or sodium bicarbonate the rate of gastric emptying was increased relative to that of water. However, at concentrations above about 200 mN these salts slowed gastric emptying in proportion to their concentration. To explain these facts it was suggested that sodium ions moved into the sensitive vesicle by some special mechanism susceptible of saturation, chloride and bicarbonate and water following in the interest of electrical and osmotic equilibrium: these solutes in concentrations up to the saturation level therefore caused the sensitive vesicle to swell, thus decreasing the inhibitory signal which was postulated to slow emptying even with meals of water.

There was some experimental evidence consistent with the view that chloride ions did passively enter the postulated osmoreceptive vesicle. Over the range of concentration from 0–70 to 100 mN the rate of emptying of test meals containing ammonium chloride was independent of the concentration of solute. As ammonium ion and chloride ion are known to penetrate the red-cell membrane, the ammonium in the form ammonia, it seemed possible that it and the chloride ion both penetrated the sensitive vesicle of the receptor and thus had no osmotic action. On the other hand, ammonium sulphate in test meals, unlike ammonium chloride, slowed gastric emptying. This distinction was ascribed to the non-penetration of the sulphate ion into the receptor, with a corresponding limitation of movement of ammonium. Consistent with this, sodium sulphate was effective at lower concentrations than sodium chloride in slowing gastric emptying, presumably because of the postulated non-penetration of the sulphate ion into the sensitive vesicle of the osmoreceptor.

The present series of results shows a consistent difference between the effects of these four solutions containing citrate ion upon the emptying of the stomachs of the five subjects. The differences might be caused by

(1) the variations of the concentration of the three ions of citrate in the meals;

(2) the variations of the sodium concentration in the meals;

(3) the variations of the pH of the meals; or

(4) the variations of the concentration of titratable acid in the meals.

In the results from the present experiments the response to trisodium citrate was similar to that previously found for sodium chloride. The response to ammonium citrate was similar to that for ammonium chloride, in that the volume of meal recovered was independent of concentration of solute. It is therefore inferred that the citrate ion with three negative charges penetrates the osmoreceptor in a manner similar to the chloride ion in this context. That is to say, it passes freely and exerts no osmotic effect on the duodenal receptor. Thus the variation in concentration of citrate ion cannot be considered as directly responsible for the effect upon gastric emptying. With solutions of disodium citrate, monosodium citrate and citric acid the citrate ions will on an average be carrying less than three negative charges, with the possible implication that these ions will penetrate at least as readily as those carrying three charges. If this were so, the different actions of the various salts could not be attributed to the simple osmotic actions of the ions.

The results for test meals containing ammonium chloride or ammonium citrate have a limitation. There is a concentration, which varies from subject to subject, above which the meals are nauseating. When nausea occurs in these subjects gastric emptying is slowed. Such results and those for higher concentrations have been excluded from consideration because they have caused nausea, but the decision as to the occurrence of nausea is a difficult one to make. For this reason the experiments may possibly be considered biased in terms of producing results consistent with the view that ammonium chloride and citrate do not slow gastric emptying. The conclusion about meals containing ammonium chloride and ammonium citrate has, therefore, to be that they do not influence gastric emptying over a rather narrow range of concentration.

Concerning the nausea, it might be suggested that the sensation arises as a result of the slowing of gastric emptying or that the sensation arises and the slowing of gastric emptying occurs as a product of the sensation. This second hypothesis is probably preferable, since fats and sugars can cause considerable slowing of gastric emptying without co-existent nausea, whereas much less slowing of gastric emptying by ammonium chloride or ammonium citrate is associated with nausea. This does not, of course, necessarily exclude the first hypothesis from consideration.

It is clear from Fig. 1 that an increase in the concentration of citrate ion as the dihydrogen sodium salt is more effective in slowing gastric emptying than the same increase in concentration of citrate ion as the trisodium salt. This cannot be attributed simply to the differences in concentration of

sodium ion, since the concentrations of sodium ion are, of course, less for equivalent concentrations of the monohydrogen and dihydrogen citrates than they are for trisodium citrate. With sodium citrate the maximal rate of emptying is at about 50–100 mN sodium ion; with dihydrogen sodium citrate the concentration of sodium is 100 mN when the total concentration of citrate is 300 mN, which can be seen from Fig. 1 to give a considerable slowing of gastric emptying relative to water. Thus the major variations in emptying cannot be ascribed to the sodium ion.

It is also unlikely that the pH of the test meal is the factor deciding the rate of emptying. With dihydrogen sodium citrate a change in concentration from 50 mN, pH 3·8, to 250 mN, pH 3·7, produces in the results of HT an increase in volume recovered from 260 to 540 ml., i.e. 280 ml. for a change of 0·1 pH unit. A change from 250 mN hydrogen disodium citrate, pH 5·0, to 250 mN dihydrogen sodium citrate, pH 3·7, a change of 1·3 pH units, is associated with an increase in volume recovered from 320 to 540 ml., that is, 220 ml., which is less than that produced for a change of 0·1 pH units with dihydrogen sodium citrate.

As an alternative to some osmoreceptor system directly sensitive to a wide range of concentrations of constituents in the duodenal lumen, there is the possibility of a system involving the action of the pancreatic and duodenal secretions in neutralizing acid. It might be suggested that when as a result of the entry of gastric contents the pH of the duodenal contents fell below, say, pH 6, effective gastric propulsive activity would cease as a result of the stimulation of a duodenal receptor. The acid should cause the release of secretin and the pancreas and Brunner's glands would secrete bicarbonate. The acid in the duodenum would be neutralized and the inhibition of gastric emptying would stop. A further portion of gastric contents would be transferred to the duodenum and the process would repeat itself.

With such a system the rate of emptying of very acid test meals would depend upon the rate of secretion of bicarbonate by the pancreas and duodenum, the amount of acid transferred from the stomach to the duodenum being equal to the rate of secretion of bicarbonate. Thus, as meals with successively higher concentrations of acid were given, a fall in the volume transferred to the duodenum in unit time should result in a constant rate of delivery of acid to the duodenum.

With such a system the rate of transfer from the stomach to the duodenum of solutions of weak acids and strong acids of equal titratable acidity should be similar and within limits independent of the pH of the solution. The data on the gastric emptying of solutions of citric acid, dihydrogen sodium citrate and of hydrogen disodium citrate are suitable for testing this hypothesis, since the respective pH values of the solutions

are about 2·4, 3·7 and 5·0, a range which should be sufficient to show up any dependence of gastric emptying on the pH of the gastric contents.

To test the view that the rate of gastric emptying is such as to deliver a fixed amount of 'titratable acid' to the duodenum during the test, it is necessary to postulate some threshold for the receptor detecting the acidity of the duodenal contents. The findings of Thomas & Crider (1940) may be relevant: their threshold for the release of secretin was not below pH 5·0.

From the present results for hydrogen disodium citrate it appears that the receptor mechanism adjusts its response to the concentration of solute at pH values above 5. As a first approach an assumption that the threshold for the inhibition of gastric emptying is effectively pH 6 has been found to explain the present results, as will be shown below.

Titration curves for various concentrations of citric acid against sodium hydroxide were made, and from these the titratable acidity up to pH 6·0 in m-equiv/l. of any of the meals containing citrate could be determined. From the data of Fig. 1 the volume of the test meal remaining for a particular concentration of solute was determined. This value, subtracted from the initial volume of the meal, 750 ml., gave the volume of the meal transferred to the duodenum in the standard time. The volume transferred multiplied by the titratable acid to pH 6·0 for the particular meal gave the amount of titratable acid transferred to the duodenum in the standard time. The results of such calculations for the meals containing citric acid, dihydrogen sodium citrate or hydrogen disodium citrate are shown in Fig. 2. For comparative purposes the results are all shown as amount of transfer in 15 min, those from meals lasting 10 min or 20 min having been corrected by multiplying by 3/2 or 3/4, respectively. It may be seen that the steady rate of transfer of titratable acid to the duodenum was between 20 and 30 m-equiv/15 min. It was substantially independent of whether titratable acid was in the form citric acid (pH about 2·4), sodium dihydrogen citrate (pH about 3·6) or hydrogen disodium citrate (pH about 5·0). These results are therefore incompatible with the direct control of gastric emptying by the pH of the gastric contents acting in the duodenum, but consistent with the concept of control somehow related to the amount of titratable acid transferred to the duodenum. If the threshold of the supposed duodenal receptors is set at pH 5 instead of pH 6, it is found that the calculated transfer to the duodenum of acid titratable to pH 5 is greater for the meals containing citric acid or monosodium dihydrogen citrate than it is for meals of disodium monohydrogen citrate. Only when the threshold of the receptor is set at about pH 6 can the results be explained on the present hypothesis. It may be seen that in the results for JS there is evidence that the steady rate of transfer of acid in the form

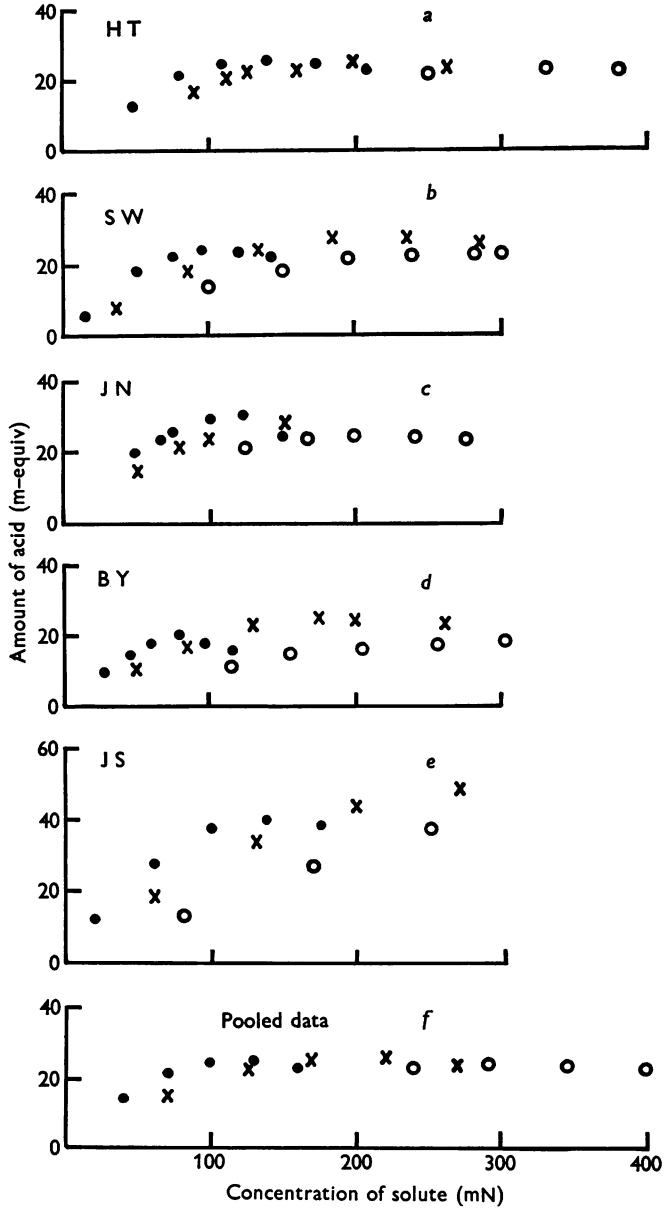

Fig. 2. Amounts of titratable acid, measured to pH 6, transferred to the duodenum in 15 min. ●, citric acid; ×, dihydrogen monosodium citrate; ○, monohydrogen disodium citrate. Ordinates, amount of titratable acid (m-equiv) leaving stomach in 15 min; abscissae, concentration of solute (mN). *f* shows pooled results.

dihydrogen sodium citrate or hydrogen disodium citrate was not reached, possibly because the duration of tests in this subject was only 10 min.

The gastric contents transferred to the duodenum contain not only the acid given by mouth, but the acid secreted by the stomach. Results on this point were obtained for these meals. The maximal amount secreted in any subject was about 5 m-equiv in the period of the test. About a quarter or less of this acid was transferred to the duodenum during the test. Thus even in the subject who secreted the most acid an error of only about 1·25 m-equiv is introduced into the calculation of the amount of acid transferred to the duodenum by neglecting the acid secreted by the stomach. In the other subjects the error is less.

Because the rates of emptying of solutions containing ammonium chloride or ammonium citrate were independent of the concentration over a limited range of these salts, it was concluded tentatively that the citrate ion and the chloride ion passed freely into the hypothetical osmoreceptor. However, this conclusion must raise a reservation about the similarity between the membrane of the osmoreceptor and the red cell, since red cells are not haemolysed in solutions of ammonium citrate. It would not be expected that a relatively large hydrated ion such as the citrate ion would penetrate as freely as chloride. However, comparison of results for HT in Hunt & Pathak (1960) and in the present paper (Table 1) is consistent with the view that chloride and citrate as sodium salts have very little action on the hypothetical osmoreceptor over a wide range of concentrations. There is therefore nothing to suggest that the gastric emptying of titratable acid with the test meals of dihydrogen sodium citrate is limited by the presence of citrate ion. As may be seen from the pooled results of Fig. 2, the general statement that the transfer of an acid meal from the stomach to the duodenum with citric acid and with salts of citric acid is independent of the pH of the meal, is well founded.

The views advanced here fit into a more comprehensive scheme for the analysis of the control of gastric emptying of acid solutions proposed by Lagerlöf, Rudewald & Perman (1960). In their experiments the volume of the gastric contents was more or less constant at 250 ml. and the transfer of acid to the duodenum was about half that calculated from the present data, with pH 6 as the end-point. They were able to show that the rate of bicarbonate secretion into the duodenum was linearly related to the amount of acid entering the duodenum from the stomach. They gave grounds for thinking that the maximal concentration of bicarbonate in the duodenal secretions is about 120 mN. Our subject JS was calculated to have transferred about 40 m-equiv of acid to the duodenum in 15 min, which would require more than 1 l. of duodenal secretions for neutraliza-

tion, which is just about feasible (Wilhelmj, Sachs, Slutzky & Barak, 1950). However, Lagerlöf *et al.* (1960) found evidence of diffusion of hydrogen ion through the duodenal wall which might be relevant in our context. It is also noteworthy that in JS the tests lasted only 10 min, so that at the time of recovery there may have been a quantity of acid in the duodenum not yet neutralized, which would tend to exaggerate the amount of neutralization occurring per unit time in the duodenum.

In an earlier study of SW, JS and HT (Hunt & Pathak, 1960) with solutions containing various concentrations of sodium chloride and 20 mN hydrochloric acid there was in each instance a curve similar in shape to that for HT with hydrogen disodium citrate. It is not clear why the humped curve is seen only with HT in the present study, but it presumably depends upon a combination of a particular pH and concentration of sodium ion in the lumen of the duodenum.

SUMMARY

1. Test meals of 750 ml. of solutions of citric acid, dihydrogen sodium citrate, hydrogen disodium citrate and trisodium citrate at various concentrations were given by tube to five subjects. Three of the subjects also received meals of ammonium chloride and ammonium citrate. The meals, which contained phenol red, were recovered after an interval fixed for each subject. The volume of original meal recovered was determined by using the amount of phenol red recovered as the index of the volume of original meal remaining in the stomach.

2. The rate of transfer of acid, measured by titration to pH 6·0, reached a steady rate for each subject. The rate of emptying of the gastric contents was independent of the pH of the gastric contents.

3. It is suggested that a receptor in the duodenum is excited when the duodenal contents are more acid than pH 6·0 and inhibits gastric emptying. The rate of gastric emptying is therefore set by the pancreas and duodenum, which neutralize the acid entering from the stomach.

4. The relations between gastric emptying and the concentrations of sodium citrate in the meal were similar to those for sodium chloride, the rate of emptying with concentrations up to 100 millinormal being more rapid than the emptying of water.

5. The rate of emptying of solutions of ammonium citrate and of ammonium chloride was independent of concentration. It is concluded that the citrate ion has little influence on receptors regulating gastric emptying.

It is a pleasure to acknowledge the co-operation and persistence of the students who were subjects in these experiments. I am indebted to Miles Laboratories Inc., Elkhart, Indiana for a generous supply of citric acid and its salts.

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