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THE SLOWING OF GASTRIC EMPTYING BY FOUR STRONG ACIDS AND THREE WEAK ACIDS

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SUMMARY

1. Test meals of 750 ml. water containing a range of concentrations of hydrochloric, nitric, sulphuric, cyclamic (cyclohexylsulphamic), tartaric, L-ascorbic and citric acids were instilled into the stomachs of sixteen healthy subjects.

2. The greater the concentration of an acid in the instilled meal, the greater was the volume of meal recovered after a fixed interval.

3. For the weak acids, tartaric, ascorbic and citric, the concentrations which gave a recovery of 450 ml., out of the 750 ml. instilled, increased with increase in the molecular weight of the acid.

4. For the strong acids, hydrochloric, nitric, sulphuric and cyclamic, the concentrations which gave a recovery of 450 ml., out of the 750 ml. instilled, were approximately equal. These concentrations were less than those for weak acids.

5. The strong acids with high molecular weights were slightly more effective in slowing gastric emptying than were those with low molecular weights.

6. The approximate equality of effectiveness of nitric, sulphuric and cyclamic acids to hydrochloric acid is attributed to the presence of chloride ion in the duodenal contents. Thus all the strong acids instilled produce an environment of hydrochloric acid around the receptor.

7. A model for a duodenal receptor responding to acids is proposed.

INTRODUCTION

It has been known since the beginning of this century that hydrochloric acid added to test meals slows gastric emptying (Hedblom & Cannon, 1909). This effect is believed to be mediated by duodenal receptors which are stimulated by the test meal as it leaves the stomach (Shay & Gershon-Cohen, 1934). These receptors inhibit the peristaltic activity of the stomach

(Thomas, Crider & Mogan, 1934), and possibly increase the resistance of the duodenum to filling (Weisbrodt, Wiley, Overholt & Bass, 1969).

The greater the concentration of hydrochloric acid in a test meal the greater is the slowing of gastric emptying. This finding is consistent with the notion that the pH or the concentration of hydrogen ions in the gastric effluent determines the degree of stimulation of the duodenal receptors (Hunt & Knox, 1969). Hunt & Knox (1969) also found that for a given concentration, acids with high molecular weights were less effective in slowing gastric emptying than were those with low molecular weights. Of the nine acids they tested, all except hydrochloric acid were weak acids with pK values greater than 2·1. However, the results with hydrochloric acid were consistent with those for weak acids. For example, acetic acid (mol. wt. 60), which is very weak, was almost as effective as hydrochloric acid (mol. wt. 36·5), in spite of the hydrogen ion activity being at least 100 times greater with hydrochloric acid than with acetic acid. This finding forced Hunt & Knox (1969) to discard the notion that the duodenal receptors were responding simply to the pH of the test meal as it left the stomach. From previous work (Hunt & Pathak, 1960) it was apparent that the slowing effect of most salts on gastric emptying was of a minor order compared with that of acids even though the anions were common. Thus the duodenal receptors studied by Hunt & Knox (1969) were truly responding to acid. Their comparision of eight weak acids with hydrochloric acid was not entirely satisfactory since the molecular weight of the hydrochloric acid was less than those of all the other acids. What was needed was a set of strong acids with a range of molecular weights spanning that of the weak acids. Fortunately there are three such strong acids which can be given to man, nitric acid (mol. wt. 63) sulphuric acid (mol. wt. 98) and cyclamic acid (mol. wt. 179). In order that weak and strong acids should be studied in the same group of subjects, tests on the previously studied tartaric and citric acids were repeated. One new weak acid, ascorbic, was included in the tests.

The present study is a comparison of the action of weak and strong acids on the receptors slowing gastric emptying.

METHODS

The methods used were the same as those described by Hunt & Knox (1969). Briefly, 750 ml. test meals, containing various concentrations of different acids and phenol red as a marker, were instilled into fasting subjects after their stomachs had been washed out with 250 ml. tap water.

After an interval, fixed for each subject, but varying between 10 and 25 min for different subjects, the gastric contents were withdrawn and the stomach washed out again. From the amount of phenol red recovered, the volume of original meal recovered was computed. Phenol red is not adsorbed or absorbed by the stomach of man (Ivey & Schedl, 1970) even at pH 1·0.

Statistical methods

Linear regression equations for volume of original meal recovered against concentration of acid in the given meal were computed after checking by eye that a straight line adequately represented the results.

Because there is some uncertainty about the threshold concentration of acid required to operate the duodenal receptors, the regression lines were fitted only for concentrations of 10 mN upwards. This matter is discussed at length in a previous paper (Hunt & Knox, 1969).

The concentrations of acid required to give a recovery of 400 ml. (Table 2) or 450 ml. (Table 4) were determined from the regression equations with concentration as dependent variable. To be consistent with the earlier paper (Hunt & Knox, 1969) the constants of the alternate regression, with volume as dependent variable, are given in Tables 1 and 3.

RESULTS

Hydrochloric acid and sulphuric acid

Fig. 1 shows the volumes of 750 ml. meals recovered plotted against the concentrations of sulphuric acid in the relevant meals for subject C.L. This subject showed the usual increase in volume of meal recovered as the concentration of acid in the instilled meal was increased. The figure also indicates how the concentration giving a recovery of 450 ml., in this instance 28 m-equiv/l., was determined.

Table 1 shows the regression constants (volume as dependent variable) for the relation between volume of meal recovered after a fixed time interval and the concentration of hydrochloric acid or sulphuric acid in the instilled meal.

To compare the effectiveness of the two acids in slowing gastric emptying, the concentrations giving recoveries of 400 ml. were computed from the regression constants with concentration as dependent variable. The concentration giving a recovery of 400 ml. was chosen because this was the largest volume of recovery common to all subjects. Table 2 shows that the mean concentration of hydrochloric acid giving a recovery of 400 ml. was 25 mM, and of sulphuric acid 23 mN.

If the effectiveness of all acids were a function of their molecular weight it would be expected from Fig. 5 (Hunt & Knox, 1969) that about 35 mN sulphuric acid (mol. wt. 98) would have the same effect as 25 mN hydrochloric acid (mol. wt. 36·5). This difference of 10 mN should have been readily detected in the present experiments, since the standard error of the mean within-subject difference between the concentrations of hydrochloric and sulphuric acids giving recoveries of 400 ml. was only $\pm$ 1·55 mN. Thus sulphuric acid is anomalous since molecular weight does not determine its effectiveness.

TABLE 1. The effect of acid in test meals on the volume of meal recovered from the stomach after a fixed time interval. Relation between concentration (x mN) of hydrochloric acid and of sulphuric acid in test meals and the volume of meal (y ml.) recovered after a fixed time interval. $y = a + bx$ so that 'a' is the volume of meal computed to be recovered with zero concentration of acid. The mean volume of water recovered was determined by experiment

Subject...	K.G.	R.D.	C.L.	M.A.	B.L.	W.S.	B.N.	R.B.	C.T.	M.E.
Duration of meals (min)...	25	20	20	15	20	20	25	20	25	20
Hydrochloric										
a	36	131	181	212	108	212	175	75	314	60
b	8·60	8·74	8·33	11·30	9·00	7·46	9·53	15·48	2·57	10·76
S.E. of slope	2·08	1·91	2·14	2·31	2·03	3·71	2·49	4·23	2·44	0·58
n	17	20	22	16	15	8	14	14	12	3
Range of concentrations	10–30	10–30	10–30	10–30	10–30	10–30	10–30	10–30	10–31	10–30
Sulphuric										
a	85	180	66	194	169	310	301	223	252	210
b	7·69	5·97	13·05	5·98	5·54	3·29	4·62	5·04	5·69	6·02
S.E. of slope	2·19	1·72	1·45	1·96	3·08	2·98	2·71	3·92	2·84	3·43
n	20	19	21	18	12	9	7	11	9	9
Range	10–33	10–33	10–33	10–33	10–26	10–33	10–33	10–33	10–33	10–33
Mean volume of water remaining	110·5	158·3	190·6	163·6	240·5	330·0	308·5	224·4	386·2	240·0
S.E. of mean	19·8	23·5	18·6	25·7	64·0	33·6	24·1	41·1	19·6	23·3
n	6	9	13	9	4	4	4	5	4	9

TABLE 2. Concentrations (mN) of hydrochloric and sulphuric acids giving recoveries of 400 ml. after a fixed time interval. The results were calculated by substituting 400 ml. in the regression equation $x' = a' + by'$, where x' = concentration of acid in meal, and y' = volume of meal recovered

Subject	Hydrochloric (mN)	Sulphuric (mN)
K.G.	31·1	27·9
R.D.	25·7	26·6
C.L.	22·6	24·0
M.A.	24·2	24·8
B.L.	27·4	22·8
W.S.	21·8	19·5
B.N.	23.8	19·2
R.B.	19·9	22·2
C.T.	22·1	19·8
M.E.	31·5	21·6
Mean	25·0	22·8
S.E.	± 1·24	± 0·24

Within-subject S.E. of difference ± 1·55 mN.

Hydrochloric acid, nitric acid and cyclamic acid

Table 3 shows the relation (volume as dependent variable) between the concentrations of acids (mN) and the volumes of meal recovered for hydrochloric (mol. wt. 36·5), nitric (mol. wt. 63), sulphuric (mol. wt. 98) and cyclamic (mol. wt 179) acids. All are strong acids, the least strong being cyclamic (cyclohexylsulphamic) with a pK of less than 0·9.

Table 4 shows the concentrations of these acids computed (concentration as dependent variable) to give recoveries of 450 ml. The means for hydrochloric, nitric, sulphuric and cyclamic acids are 35·2, 34·8, 29·8 and 24·7 mN respectively. Thus to a first approximation all four strong acids are about equally effective in slowing gastric emptying. There is certainly no decrease in effectiveness with molecular weight as there is with weak acids.

Amongst these strong acids there is a tendency for those with the higher molecular weights, sulphuric and cyclamic, to be slightly more effective than hydrochloric acid and nitric acid. To put this on a quantitative basis a regression equation was computed relating molecular weight to the concentration of acid giving a recovery of 450 ml. To eliminate the considerable between-subject variation, the concentration of each acid giving a recovery of 450 ml. was expressed as a percentage of the mean concentration of the four acids giving 450 ml. recoveries for that subject. The regression was found to be

$$x = 119 - 0{\cdot}197y, \quad \text{S.E. of slope} = \pm 0{\cdot}063, \quad P = 0{\cdot}005,$$

where

x = concentration of acid giving 450 ml. recovery, as % mean for four acids in each subject:

y = molecular weight of the strong acid.

The significant negative slope in the regression for these results indicates that with increased molecular weight these four strong acids became more effective in slowing gastric emptying.

TABLE 3. The effect of acid in test meals on the volume of meal recovered from the stomach after a fixed time interval. Relation between concentrations (x mN) of acid in test meals and the volume of meal (y ml.) recovered after a fixed time interval. $y = a+bx$ so that 'a' is the volume of meal computed to be recovered with zero concentration of acid. The mean volume of water recovered was determined by experiment

Subject	H.T.	B.G.	W.Y.	F.S.	W.D.	E.S.	E.E.
Duration of meals (min)...	20	20	20	20	20	20	20
Hydrochloric							
a	82	30	184	280	49	105	499
b	6·37	10·90	6·63	5·92	9·72	9·13	7·81
S.E. of slope	1·71	4·25	1·63	2·36	1·92	2·87	17·59
n	10	6	12	10	11	8	5
Range	23–50	29–40	18–40	8–41	8–40	8–40	14–23
Cyclamic							
a	118	−8·5	258	107	215	94	185
b	8·20	17·27	5·98	16·14	8·08	11·81	11·73
S.E. of slope	1·16	4·81	1·97	8·10	5·57	3·44	1·50
n	12	7	8	9	13	10	12
Range	10–67	3–25	10–28	10–26	8–30	10–30	0–30
Citric							
a	—	−24	263	293	−55	329	202
b	—	3·84	1·55	1·77	4·03	1·52	3·20
S.E. of slope	—	0·71	0·79	0·40	1·11	0·84	0·59
n	—	4	6	8	6	5	5
Range	—	51–151	51–151	45–151	51–127	45–151	45–127
Ascorbic							
a	162	184	326	223	96	308	—
b	1·81	2·86	1·82	3·00	2·80	1·95	—
S.E. of slope	0·44	0·97	0·59	0·56	0·56	1·43	—
n	18	7	9	12	13	7	—
Range	10–155	28–105	33–119	12–125	13–137	18–104	—

TABLE 3 (*cont.*)

Subject	H.T.	B.G.	W.Y.	F.S.	W.D.	E.S.	E.E.
Duration of meals (min)...	20	20	20	20	20	20	20
Tartaric							
a	—	155	164	183	177	161	—
b	—	3·56	3·95	4·33	3·22	4·43	—
S.E. of slope	—	1·50	2·43	1·46	1·15	2·14	—
n	—	7	5	5	6	6	—
Range	—	26–86	38–73	38–73	38–88	26–73	—
Nitric							
a	95	90	—	152	—	203	—
b	7·65	6·39	—	7·43	—	4·44	—
S.E. of slope	1·27	3·40	—	1·87	—	1·58	—
n	13	12	—	10	—	13	—
Range	5–36	6–39	—	5–36	—	1–29	—
Sulphuric							
a	109	69	176	462	226	147	499
b	6·89	7·67	6·42	4·51	4·67	7·80	7·82
S.E. of slope	2·46	2·18	2·62	8·76	2·77	4·91	17·6
n	10	6	13	10	12	7	5
Range	10–35	20–62	14–38	17–29	10–38	17–38	14–23
Mean volume of water remaining	145	147	192	211	173	204	180
S.E. of mean	—	25·5	19·3	20·2	30·3	36·6	11·0
n	—	8	6	10	13	8	3

Tartaric, ascorbic and citric acids

Table 3 shows the regression constants (volume as dependent variable) describing the relations between the concentrations of tartaric, ascorbic and citric acids and the volumes of the meals recovered.

From these regression equations the concentrations of tartaric acid (mol. wt. 150) and citric acid (mol. wt. 192) giving recoveries of 450 ml. were computed for each subject. For each subject these two concentrations were plotted against the molecular weights of the respective acids. From a straight line connecting these two points the concentration of L-ascorbic acid (mol. wt. 176) expected to give a recovery of 450 ml. was read off. The mean of the values expected for ascorbic acid was 92 mN; the mean of the observed values was 83 mN (within-subject S.E. ±7·6). The difference between the expected and observed values is not statistically significant.

TABLE 4. Concentrations of acid (mN) in test meals giving recoveries of 450 ml. after a fixed time interval. n = number of observations included in the mean. The concentrations were calculated by substituting 450 ml. in the regression equation $x' = a' + by'$ where x' = concentration of acid in meal and y' = volume of meal recovered. The value in parentheses for E.E. was computed when the results for meals of water were included in the regression. For all other subjects the results for the meals of water were omitted. The three values for n are given to allow comparisons to be made. * Computed from Table 1 (Hunt & Knox, 1969)

Subject		Ascorbic	Citric	Cyclamic	Hydro-chloric	Sulphuric	Tartaric	Nitric
B.G.		83·9	122·1	19·2	37·4	45·7	72·3	31·5
H.T.		116·1	114·0*	38·1	49·1	35·1	68·7*	40·9
F.S.		71·4	90·4	19·4	28·5	21·7	60·1	33·8
E.S.		50·9	89·0	26·7	34·6	29·5	59·1	32·9
W.Y.		68·1	112·4	27·9	36·5	30·4	63·5	—
W.D.		105·3	117·6	20·1	38·2	27·8	77·0	—
E.E.		—	78·0	(21·6) 30·9	21·9	18·8	—	—
Mean	n 7	—	103·4	24·7	35·2	29·8	—	—
	n 6	82·5	107·6	25·2	37·4	31·7	66·8	—
	n 4	—	—	25·9	37·4	33·0	—	34·8

Thus the results for ascorbic acid fit into the pattern found for the other weak acids.

DISCUSSION

Figs. 1 and 2 summarize our information about the action of different acids in slowing gastric emptying. From Fig. 1 it may be seen that on increasing the concentration of sulphuric acid in the given meal from 10 to 35 mN the recovery of the meal after a fixed interval increased from about 200 up to 500 ml. A similar effect, to different degrees, was seen with all thirteen acids examined.

The relationship between the effectiveness of acids in slowing gastric emptying and their molecular weights is shown in Fig. 2. The ordinate shows the mean concentration (m-equiv/l.) of an acid giving a recovery of 450 ml. after a fixed interval following a 750 ml. meal containing the relevant acid. The higher the value on the ordinate, the less effective was the acid in slowing gastric emptying. It may be seen that the concentrations giving recoveries of 450 ml. increase with molecular weight for acetic, propionic, lactic, butyric, hexanoic, tartaric, ascorbic and citric acids. All these acids are more or less weak, the lowest pK being that for tartaric, 3·0. Molecular weight thus seems to be the main determinant of effectiveness of weak acids in slowing gastric emptying. The relationship is stronger than appears in Fig. 2 since this includes all our results in several different

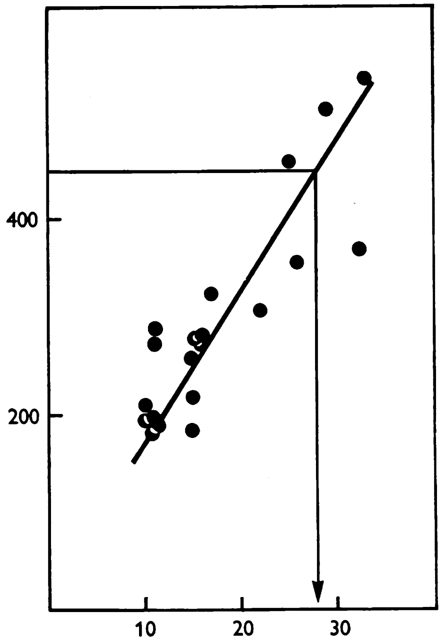

Fig. 1. The effect of increasing the concentration of sulphuric acid in instilled test meals on the volume of meal recovered after 20 min. Ordinate: volume of meal recovered at 20 min (ml.). Abscissa: concentration of acid (mN).

groups of subjects. In a previous paper (Hunt & Knox, 1969) the corresponding correlation coefficient for six acids compared in fourteen subjects was 0·997.

Fig. 2 shows that the results for three strong acids, nitric, sulphuric and cyclamic, stand apart from those for weak acids with similar molecular weights. In spite of their higher molecular weights these three strong acids are at least as effective as hydrochloric acid.

Is acid the stimulus to the receptor?

That acetic acid, a weak acid (pK 4·8), and hydrochloric acid, a very strong acid, should be nearly equal in stimulating a receptor allegedly responding to acid, is remarkable. That in addition the molecular weights of acids, which is entirely a property of their anions, should dominate the responses of the receptor to different acids must raise the question as to whether or not the receptor is truly responding to acid. In concentrations up to 125 mN, solutions of sodium chloride or sodium citrate do not slow gastric emptying, in fact they leave the stomach about twice as quickly as water (Hunt & Pathak, 1960; Hunt & Knox, 1962). Moreover, there are results for calcium, potassium and ammonium salts which show that most anions have only relatively weak actions on gastric emptying (Hunt & Pathak, 1960). There seems to be no escape from the conclusion that the receptor studied in the present work responds to acid.

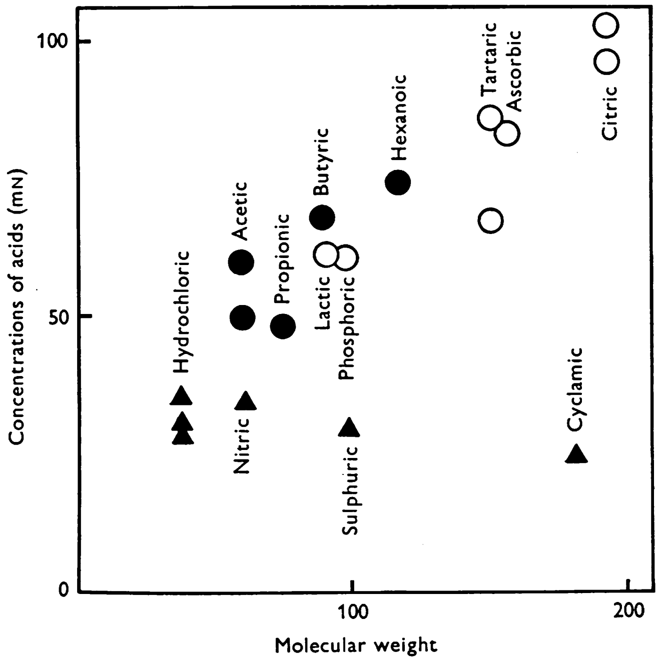

Fig. 2. The relation between molecular weights of weak and strong acids and their concentration in 750 ml. test meals which gave recoveries of 450 ml. from the stomach after a fixed interval. For hydrochloric, acetic, tartaric and citric acids there is more than one point because the experiments were replicated in different groups of subjects. Ordinate: concentration of acids (mN). Abscissa: molecular weight.
Strong acids (▲): hydrochloric, nitric, sulphuric, cyclamic. *Fatty acids* (●): acetic, propionic, butyric, hexanoic. *Other acids* (○): lactic, phosphoric, tartaric, ascorbic, citric.

The receptive process as analogous to titration

It would be easy to envisage a duodenal receptor responding to acid much like a glass electrode. A potential across a cell membrane varying with the concentration gradient for hydrogen ion (strictly H_3O^+), could serve as a generator potential for a neural mechanism. However, such a simple receptor could not give almost equal graded responses proportional to the concentration of hydrochloric acid and proportional to the concentration of acetic acid. A change from 10 to 100 mN with hydrochloric acid would give nearly a tenfold increase in hydrogen ion activity from pH 2·0 to 1·1. But for acetic acid such a change in concentration would give only a threefold increase in hydrogen ion activity, from pH 3·5 to 3·0, because acetic acid dissociates feebly. Yet the receptor responses to these two acids have the ratio 1·6 to 1, which is accounted for by the ratio of their molecular weights (1·6 to 1).

Given solutions of a variety of acids with different pK values the simplest way to determine their concentrations in m-equiv/l. is to titrate aliquots and the model proposed for the duodenal receptor responding to acids is assumed to work by a process analogous to titration.

The end-point of the titration process

Fig. 2 shows on the ordinate the mean concentration of each of thirteen acids which gave recovery of 450 ml. after a fixed interval.

These concentrations were all measured by titrating the acids to pH 6·5. Titrating to any substantially different pH would give neither a rectilinear nor an orderly relationship between concentrations which gave recoveries of 450 ml. and molecular weights. For example, it makes little difference to the recorded concentration of hydrochloric acid whether it is titrated to pH 3, pH 4, etc. However, for acetic acid, at pH 3 the titration of its proton donors has scarcely begun and even at pH 5·8 the titration is not complete, the pK of acetic acid being 4·8.

The decision to titrate the acids in the test meals to pH 6·5 was taken during experiments with citric acid and its sodium salts because it resulted in apparent equal deliveries to acid to the duodenum (Hunt & Knox, 1962). The same end-point was used for the titration of other acids studied by Hunt & Knox (1969). As pointed out above, it makes little difference with the majority of acids used in these experiments whether they are titrated to pH 6 or pH 8 since the titration is virtually complete by pH 6. On the other hand, phosphoric acid has three pKs (2·1, 7·2, 12·7), so that changes in the range pH 6–8 cause the dissociation of acid groups and measurable quantities of base have to be added to bring about this dissociation. It thus makes a considerable difference in determining the concentration of phosphoric acid whether it is titrated to pH 6 or to pH 8. By experiment we found that the point for phosphoric acid (mol. wt. 98) in Fig. 2 fell on the line for the weak acids when the phosphoric acid was titrated to pH 6·5, at which pH about 45 % of the proton donors have dissociated. This amounts to saying that the point for phosphoric acid in Fig. 2 can be manipulated on to the line for the other weak acids by specifying that the end-point for all the titrations is pH 6·5. We therefore conclude that our model should be specified as titrating to pH 6·5 to account for the results in Fig. 2.

The lack of effect of oil/water partition coefficient on the effectiveness of acids

It seems possible that acids might stimulate a receptor by penetrating a cell membrane and then increasing the activity of an enzyme by the fall in intracellular pH. Since cell membranes are thought of as being lipoprotein, acids soluble in such membranes would be expected to penetrate the membrane readily. If this were so, fat soluble acids, especially weak acids in the uncharged state, would be more effective than acids insoluble in fat. The finding that the points for acetic, propionic, butyric and hexanoic acids, with benzene/water partition coefficients varying from 0·02 to 38 (Brown & Bury, 1923), fall equally about the line in Fig. 2 for more polar acids indicates that fat solubility does not confer increased effectiveness on an acid. Our current interpretation is that the acids can affect the receptor element without themselves diffusing through a lipid membrane.

The lack of effect of the number of replaceable hydrogen ions in acids

In Fig. 2 the values on the ordinate are plotted in m-equiv/l. If the concentrations which gave recoveries of 450 ml. were specified in m-mole/l. the Figure would lose its orderliness. The value on the ordinate gives twice the weight to an acid with two replaceable hydrogen ions as that which it gives to an acid with one. This is a further point consistent with the notion that the receptor responds to acid, not to anions. Nevertheless, it is the molecular weight of its anion which determines the effectiveness of an acid after the inequality of the acids has been eliminated by recording their concentrations in m-equiv/l.

For weak acids, and the majority of the results in Fig. 2 are for weak acids, it is presumably mainly molecules of undissociated acid which move towards the receptor. However, arriving at the receptor, lactic acid gives rise to one hydrogen ion while citric acid gives rise to three hydrogen ions. Expressing concentrations as m-equiv/l. eliminates the distinction between acids with different numbers of replaceable protons.

Findings consistent with diffusion to the titration site

In man. Fig. 2 shows that the higher their molecular weight the less effective are weak acids in slowing gastric emptying, which is consistent with the effect of an acid being limited by its rate of diffusion to a hypothetical titration site.

Since Fig. 2 relates to concentrations of different acids giving equal slowing, it will be assumed that equal fluxes of different acids to the titration site are given by the equi-active concentrations on the ordinate. It is worth remarking that these different concentrations (C_{450}) are measured at a time when the intragastric volumes and the volumes that have passed into the duodenum (750–450 ml.) are the same for each acid. Assuming, for reasons given above that the concentration of acid at the titration site is zero, the steady state flux to the site is given by

$$J_{450} = DC_{450}\,A/X, \qquad (1)$$

where

J_{450} = flux giving recovery of 450 ml., assumed equal for all acids,
C_{450} = concentration of an acid giving a recovery of 450 ml.,
D = diffusion coefficient for the acid,
A = area for diffusion, and
X = length of diffusion channel.

Since A/X is assumed constant within an individual, it does not influence ratios of fluxes and may be eliminated.

As we have no precise information about the concentration of acid at the duodenal end of the diffusion channels we assume that it is linearly related to the concentration, C_{450}, in the given meal when the volume recovered is 450 ml.

There are apparently very few diffusion coefficients for acids available in the literature. We have assumed that the relation between diffusion coefficient, D, and mol. wt. (MW), following Graham's Law has the form

$$D = y\,\frac{1}{MWp} \quad \text{where } y \text{ and } p \text{ are constants.} \qquad (2)$$

Substituting in (1) for D and rearranging we obtain

$$\frac{1}{C_{450}} = \frac{y}{J_{450}}\cdot\frac{1}{MWP}.$$

Taking logarithms gives a relationship with the form

$$\log\frac{1}{C_{450}} = \log\frac{y}{J_{450}} + p\log\frac{1}{MW},$$

where y and J_{450} are constants and y/J_{450} is a constant, so that a value for p could be conveniently obtained by plotting log $10^3/C_{450}$ against log $10^3/MW$ using all our results for hydrochloric acid and the seven weak acids which we have studied. The results for phosphoric acid were excluded because the concentration giving 450 ml. was made to fit the other results in the interest of determining the pH to which the receptor appears to titrate. The results for propionic acid were excluded because it was anomalous in our studies both as an acid and as a salt (Hunt & Knox, 1968). The value for p, 0·49, obtained as the slope of the log log plot was then used to construct Fig. 3, the arithmetic plot of

$$\frac{10^3}{C_{450}} \quad \text{against} \quad \frac{y}{J_{450}}\cdot\frac{10^3}{MW^{0\cdot49}}.$$

The fairly close fit of our experimental points to the line ($r = 0{\cdot}97$) is consistent with C_{450} being determined by the coefficient of diffusion calculated on the basis of eqn. (2).

The use of C_{450} for the comparison of the effectiveness of acids is open to the objection that the choice of recoveries of 450 ml. is arbitrary. We have therefore made calculations corresponding to those leading up to Fig. 3, but using the slopes (S) of the lines relating the concentrations of acids to recoveries of meal, as in Fig. 1, against $1/MW^P$, giving a value for P of 0·65. Fig. 4 shows the fit ($r = 0{\cdot}95$) of the mean values for S for all our eight results plotted against $1/MW^{0{\cdot}65}$. These results and two other values of p

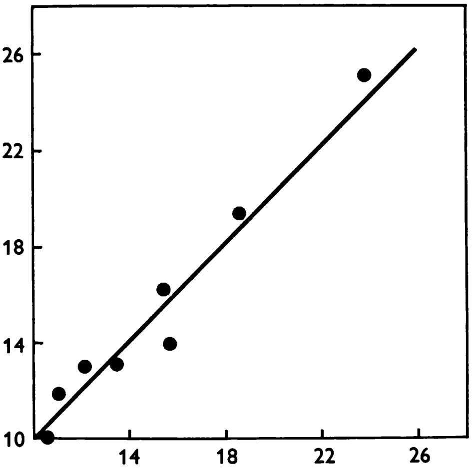

Fig. 3. Relation between mean concentrations of hydrochloric acid and seven weak acids giving recoveries of 450 ml. and their molecular weight (MW). Ordinate: 10^3/mean concentrations of acids giving recoveries of 450 ml. Abscissa: 4·7 ($10^3/MW^{0{\cdot}49}$).

obtained from plots of diffusion coefficients, taken from the literature, against $1/MW^P$ have been collected in Table 5. It may be seen that all four values of p so obtained are similar, which is consistent with the notion that diffusion is a rate-limiting step in our system.

Our mean values for acetic and citric acid for C_{450} and S, the slope of Fig. 1, are based on some hundreds of test meals in twenty subjects for each acid. For these acids there are published diffusion coefficients. The diffusion hypothesis requires that the product $C_{450} \times$ diffusion coefficient and the quotient

$$\frac{S \text{ (the slope of Fig. 1)}}{\text{diffusion coefficient}}$$

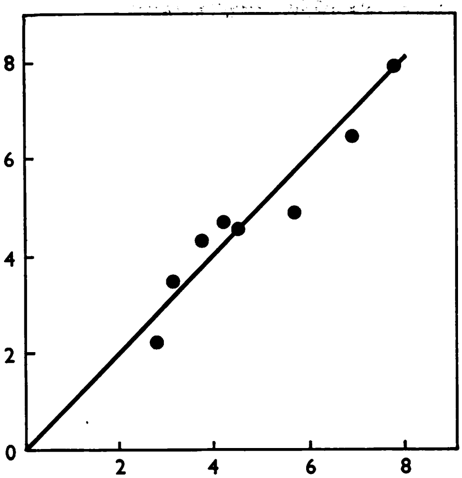

Fig. 4. Relation between mean slopes of volume recovered on concentrations of acids in the meals for hydrochloric acid and seven weak acids. Ordinate: slope of volume recovered on concentration of acid in meal (increase in volume recovered/m-equiv. l. increase in concentration of acid in meal). Abscissa: 0·206 ($10^4/MW^{0·65}$).

TABLE 5. A comparison of the power function (p) of mol. wt. (MW) obtained by assuming that diffusion coefficients (D) and indices of effectiveness of acids in slowing gastric emptying (C_{450}) are related to MW by a relationship of the form $D = K(1/MW^p)$.

D refers to diffusion coefficients taken from *Handbook of Chemistry and Physics*, 51st edn. 1970/71.

C_{450} refers to the concentrations of acids giving recoveries of 450 ml. under standard conditions.

S is the slope of the line relating concentrations of acid in meals to the volumes of meals recovered under standard conditions. The values of p do not differ significantly

Method	$p \pm$ S.E. (standard error of regression constant)
$\frac{1}{C_{450}}$ *vs.* $\frac{1}{MWP}$	0·493 ± 0·052
S *vs.* $\frac{1}{MWP}$	0·649 ± 0·085
D *vs.* $\frac{1}{MWP}$ (MW 75–192)	0·55 ± 0·05
D *vs.* $\frac{1}{MWP}$ Acetic and citric acids	0·55

be constant for different acids as shown below:

C_{450} acetic acid × diffusion coefficient $= 52 \times 1{\cdot}22 \times 10^{-5} = 63{\cdot}4 \times 10^{-5}$,

C_{450} citric acid × diffusion coefficient $= 102 \times 0{\cdot}657 \times 10^{-5} = 67{\cdot}0 \times 10^{-5}$,

$$\frac{S \text{ (acetic acid)}}{\text{diffusion coefficient}} = \frac{5{\cdot}00}{1{\cdot}22 \times 10^{-5}} = 4{\cdot}10 \times 10^{5},$$

$$\frac{S \text{ (citric acid)}}{\text{diffusion coefficient}} = \frac{2{\cdot}66}{0{\cdot}657 \times 10^{-5}} = 4{\cdot}10 \times 10^{5},$$

All these numerical values, based on C_{450} and S which are to some extent independent, are remarkably consistent with the diffusion hypothesis.

Reservations on the diffusion hypothesis

Although it seems almost self evident that the influence of molecular weight upon the effectiveness of acids must operate either through diffusion or through some form of molecular sieving, we have reservations about the quantitative treatment given above which will now be discussed. Table 5 of Hunt & Knox (1969) gives regression equations for the relation between C_{450} and mol. wt. for fourteen subjects. The slopes of these within-subject regressions vary considerably with the implication that the apparent diffusion constants for acids are not the same for all subjects. For those fourteen subjects the duration of the test was chosen in an attempt to obtain a recovery of about 200 ml. with water. This gave an opportunity to study the effect of acids over a considerable range of concentrations. It also meant that the rates of gastric emptying for different subjects during the test with water varied from 50 to 17 ml./min. However, there was no relation between the rates of emptying and the regression of C_{450} on mol. wt. There was however a correlation between the volume of meal remaining with water for a subject and his value of p in the within-subject relation

$$\frac{10^3}{C_{450}} \ vs. \ \frac{10^3}{MWP}.$$

There was also a correlation between a subject's volume of meal remaining with water and his value of p in the relation

$$S \ vs. \ \frac{1}{MWP},$$

where S is the slope of Fig. 1, with dimensions increase in volume of meal recovered/m-equiv increase in concentration of acid in the given meal. Since we as experimenters decided the volume of the test meal which would be recovered with water by adjusting the duration of the test between subjects, we have reservations at present about the implications of the close concordance of the numerical values of the computed power functions discussed above (Table 5).

In animals. B. F. Leek (personal communication) has been able to record from afferent neurones which respond to different acids applied to the abomasal mucosa in sheep. On the basis of the difference in latency with a variety of acids he concludes that free diffusion over a distance greater than 200 μ could account for his findings. This diffusion might be occurring in the gastric pits in his preparation.

An explanation of the approximate equality of action of strong acids on the receptors slowing gastric emptying

To the first approximation the slowing of gastric emptying by nitric, sulphuric and cyclamic acids was equal to that by hydrochloric acid. On reflexion it is apparent that no matter what strong acid is given by mouth, chloride ions of gastric secretion and of the secretions entering the duodenum will be present in the duodenal lumen. Suppose two parts of a solution containing 60 mN cyclamic acid is diluted by one part of pancreatic secretion containing HCO_3^- 60 mN, Cl^{-} 100 mN and Na^+ 160 mN, the resultant concentration of ions will be H^+ 20 mN, Cl^- 33 mN, cyclamate 40 mN and Na^+ 53 mN. On the assumption that ion pairs move towards the hypothetical titration site, there is sufficient concentration of chloride ions to cover the diffusion of the hydrogen ions. Thus the limit to diffusion of hydrogen ion will be mainly set by chloride ion since it is more mobile than the cyclamate ion. The receptor will therefore respond as though hydrochloric acid has been given. The more the dilution of the test meal by pancreatic secretion, the more relevant does the above explanation become since the concentration of chloride ion will rise in relation to that of cyclamate ion.

The effect of the threshold for hydrochloric acid

Hunt & Knox (1969) (Table 2) paid some attention to the question as to whether or not there was a threshold for the effect of various acids. For hydrochloric acid the results were consistent with there being a threshold at about 5–10 mN, but this threshold was not seen with the other eight acids studied, with the possible exception of lactic acid. These observations now appear to have relevance to the working of the receptor responding to acid.

It has been postulated that the receptor responds to all strong acids as though they were hydrochloric acid. But acids classed as weak, for example citric acid, give pH values as low as 2·2 at concentrations of 100 mN. This corresponds to a concentration of hydrogen ions of 6 mN. Since there is chloride ion present, it would be expected that the receptor would respond as if it were exposed to 6 mN-HCl in addition to 100 mN citric acid. Since hydrochloric acid is about three times as effective per milliequivalent per litre as is citric acid, it would be expected that the relationship between mol. wt. and effectiveness of weak acids would be distorted at high concentrations of citric acid, and other weak acids with relatively low pKs such as lactic and tartaric. This would distinguish these acids from acetic, propionic, etc., which do not give pH values less than 3 at concentrations up to 100 mN. The threshold for hydrochloric acid discussed above prevents this effect and the receptor apparently receives its acid mainly by diffusion of the undissociated weak acid, the rate of access being inversely proportional to the molecular weight of the acid.

The increased effectiveness in slowing gastric emptying of strong acids with high molecular weights

Contrary to the findings with weak acids, the strong acids with high molecular weights were more effective in slowing gastric emptying than were those with low molecular weights. The increased effectiveness of cyclamic acid might be the result of the nauseating properties of the cyclamate ion. To test this view a regression was plotted, using the results of Table 4, between the concentrations of hydrochloric, nitric and sulphuric acids (omitting cyclamic acid) giving recoveries of 450 ml. and the molecular weights of the acids. Between-subject variance was eliminated by plotting each concentration as a percentage of the mean of the three concentrations of the strong acids giving recoveries of 450 ml. for that subject. The concentration giving a recovery of 450 ml. became less as the molecular weight of the acid increased ($P < 0{\cdot}02$), just as it did when cyclamate was included. It therefore seems likely that the increase in effectiveness with molecular weight is general and not caused by any nauseating effect of cyclamate.

It is possible that the larger anions of the acids with higher molecular weights might be acting on the duodenal osmoreceptors. From the results of Hunt & Pathak (1960) and from some unpublished results of experiments with sodium cyclamate it can be stated that the increase in volume of test meal recovered/m-osmole.l. increase in concentration of sodium sulphate or cyclamate is about 1 ml. This effect is of the right order, in terms of osmoreceptor stimulation, to account for the greater effectiveness of the strong acids with high molecular weights.

A model for the receptor responding to acid

To work in the way which we have so far discovered our model of the duodenal receptor shown in Fig. 5, appears to need two parts.

1. A component which makes weak acids with high molecular weights

less effective than those with low molecular weights: a molecular sieve, already postulated for the intestinal mucosa (Fisher, 1955), or an aqueous diffusion path would serve equally well to explain our findings. Because of the quantitative agreement between the diffusion hypothesis and the results in Figs. 3 and 4, and our difficulty in specifying the properties of the sieve numerically, the concept of a diffusion path is preferred.

Assuming that the diffusion path is aqueous, on the grounds that hexanoic acid with a high fat/water partition coefficient is not especially effective in slowing emptying, it is possible to speculate on the site of the

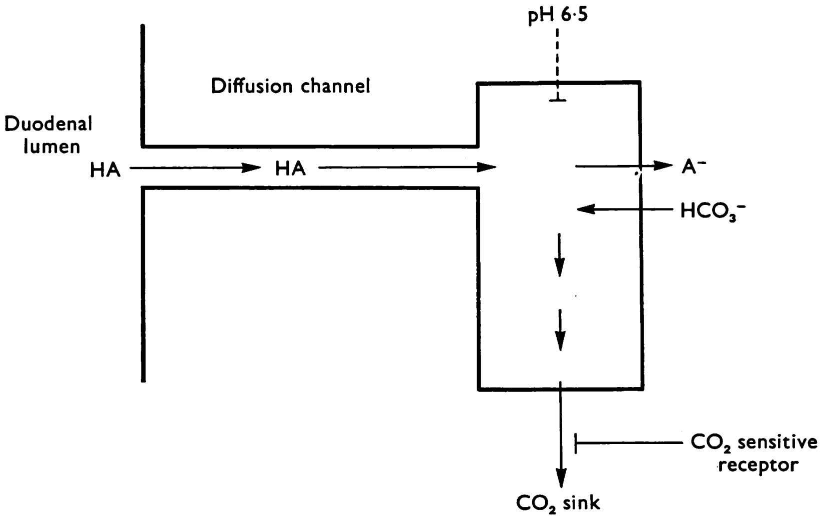

Fig. 5. Design for a hypothetical receptor responding to acids.

diffusion. To judge from the work of Harding & Leek (1972) with receptors in the abomasum of sheep, a diffusion pathway of about 200 μ would account for the difference in the latency of the afferent neuronal discharge with different acids. This is the order of magnitude of the pits in the gastric mucosa, or of the crypts of Lieberkuhn in the duodenal mucosa.

That there is a threshold concentration for the effect of hydrochloric acid might be explained by the walls of the crypts of Lieberkuhn being permeable to chloride ion so that some acid would be lost through them instead of diffusing to the titration site.

There are some facts which appear to be relevant to the walls of the diffusion pathway. Hexanoic acid has an oil/water partition coefficient of 38. Thus it should escape from the diffusion pathway if the walls were lipoprotein and should be less effective than expected on the basis of its molecular weight in the slowing of gastric emptying. In the event hexanoic acid appears to have no special properties different from other weak acids. If the cells forming the walls of the diffusion pathway held their surface at say pH 6·5 the hexanoic acid in contact with the walls would be in the charged form, hexanoate, which is not especially soluble in lipoprotein and therefore would have no special escaping tendency. On the other hand, the proposal has been made above that hydrochloric acid does escape through the walls of the diffusion pathway. To conform with the proposal for limiting the escape of hexanoate it becomes necessary to postulate that hydrogen ions in close proximity to the walls of the diffusion pathway are neutralized by the inward diffusion of say sodium bicarbonate. This is perhaps a reasonable hypothesis since many of the cells lining the crypts of Lieberkuhn are goblet cells secreting mucus; but it would not explain a threshold concentration of about 5–10 mN for hydrochloric acid yet no such threshold for the majority of the other acids tested. Another possibility is the presence of a chloride-bicarbonate exchange across the wall of the diffusion pathway. The bicarbonate would then react with the hydrogen ion in the lumen of the diffusion path and the CO_2 produced could diffuse away through the walls of the crypts. It is possible that the hydrogen ion concentration activates the supposed exchange mechanism since the mean threshold computed by extrapolation for all strong acids is 3·5 (S.E. $\pm$ 1·96) m-equiv/l. and that for all weak acids is $-3{\cdot}1$ (S.E. $\pm$ 2·02) ($P < 0{\cdot}05$).

2. A component which titrates either weak or strong acids to pH 6·5 and indicates the concentrations. Our notion is that there is diffusion of an aliquot of the duodenal contents to a titration site in the depths of the mucosa, or possibly at the deep terminations of the crypts of Lieberkuhn which are up to 500 μ from the mucosal surface (R. H. M. McMinn, personal communication). The concentration gradient to the titration site is mainly that for the molecules of the intact acids, although the titration device is titrating protons, not anions. Since the system continues to distinguish between the acids by their molecular weights, there has to be a sink into which such anions as lactate, ascorbate, tartrate and citrate diffuse. These anions when taken by mouth are either excreted in the urine unchanged or to various degrees metabolized to bicarbonate. They can be absorbed and there need be no hesitation about postulating a vascular sink for such anions moving down the diffusion pathway.

The titration site is regarded as held at pH 6·5 by bicarbonate ions entering the chamber in exchange for the anions. The bicarbonate reacts with the hydrogen ions and produces a rise in partial pressure of CO_2 which diffuses out of the chamber into a CO_2 sink. A receptor responding to P_{CO_2} lying between the titration chamber and the sink for CO_2 gives a signal which varies with the rate of generation of CO_2 at the titration site. The receptor could be analogous to those responding to CO_2 in the system controlling breathing. In essence this complexity turns all acids into carbonic acid and thus allows the response to be largely independent of the pK of the acid stimulating the receptor. Our view on the configuration of these components is shown diagrammatically in Fig. 5.

APPENDIX

Toxicity

Cyclamic acid (*cyclohexylsulphamic acid*)

At the time of these experiments cyclamic acid, as its calcium salt, was in wide use as a sweetening agent. The amounts received by our subjects were relatively small, since at the highest concentrations most of the meal was recovered. Cyclamate is now regarded as a potentially toxic substance when repeatedly ingested.

Nitric acid

The acute toxicity of nitric acid, like that of hydrochloric acid or sulphuric acid, depends mainly upon the concentration of hydrogen ion. Nitrate ion itself is relatively harmless. More than 500 m-equiv is a minimal acutely toxic dose. Thus nitric acid is as toxic as similar concentrations of endogenous hydrochloric acid. The maximal concentration of strong acids used in this work, 67 mN in HT, was less than half those used by many other workers. No untoward symptoms were produced apart from occasional nausea.

Nausea

In our experience materials which slow the gastric emptying of test meals usually produce nausea when given in high concentrations. But for corresponding action in slowing gastric emptying some substances are much more nauseating than others. For example, at relatively low concentrations glucose and potassium chloride, say 250 m-osmole/l. in test meals, slow gastric emptying equally, but at high concentrations potassium chloride is more likely to cause nausea than is glucose. When this happens the potassium chloride may appear to be slowing gastric emptying to a greater extent than glucose per m-osmole/l. In the present experiment we were studying the receptors responding to acid and slowing gastric emptying. We wished to exclude the action of any other type of receptor which might produce nausea, and slow gastric emptying. When nausea occurred we aspirated the gastric contents immediately. Cyclamic acid was the most nauseating acid and some of the subjects were excluded from the experiments because they were immediately nauseated by 10 mN cyclamic acid.

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