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THE SLOWING OF GASTRIC EMPTYING BY NINE ACIDS

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SUMMARY

1. Test meals containing a range of concentrations of nine different acids were given to twenty healthy subjects. The acids used were hydrochloric, acetic, lactic, tartaric, phosphoric, citric, propionic, butyric and hexanoic.

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

3. A rectilinear relationship between the mean concentrations of acid (m-equiv/l.) required to give a recovery of 450 ml. and the molecular weights of the acids used was found. The acids with high molecular weights were less effective than those with low molecular weights.

4. The results indicate that the molecular weights of the anions play the dominant part in determining the effectiveness of the various acids. Slowing of gastric emptying by acids does not seem to be dependent upon their pK values provided they are less than 5. The oil/water partition coefficients are also apparently irrelevant to effectiveness.

5. These findings are considered to be consistent with the view that a barrier to movement of the anions in an aqueous system limits the access of the hydrogen ions to duodenal receptors, which activate the mechanism slowing gastric emptying.

INTRODUCTION

Since the experiments of Hirsch in 1893, it has been known that acids in a meal slow gastric emptying by their action on duodenal receptors. For hydrochloric acid this has been confirmed several times (Hedblom & Cannon, 1909; Shay & Gershon-Cohen, 1934; Lorber & Shay, 1956; Hunt & Pathak, 1960), but the effects of a series of different acids have not previously been compared. The present study is a continuation of the work of Hunt & Knox (1962) who used citric acid and its sodium salts.

METHODS

The fasting subjects, nineteen students and H.T., an author, came to the laboratory at about 8.00 a.m. Following a gastric washout with 250 ml. tap water, 750 ml. of the solution of acid were given down a tube into the stomach in about 75 sec. Two small women were given only 600 ml. Either the concentration of the acid or the type of acid used was varied. Only one meal was given to each subject in any one day. After an interval of 10–30 min, constant for each subject, the gastric contents were recovered. The completeness of the recovery was then checked with a further washout with 250 ml. water. The duration of the test was chosen to give a recovery of about 200 ml. after a 750 ml. meal of water, thus allowing scope for slowing of emptying by acid.

Each litre of meal contained about 30 ml. of a saturated solution of phenol red. The concentration of phenol red in the gastric contents recovered at the end of the fixed period was used to calculate the volume of the original meal recovered (Hunt & Knox, 1962). Although some gastric absorption of phenol red from acid solutions might be anticipated (Bloom, Jacobson & Grossman, 1967) under our conditions it does not appear to have been significant (Shay, Gershon-Cohen & Fels, 1939). The short time available for emptying with a correspondingly large volume of meal relative to the area of contact with the mucosa may be the explanation. The same factors make the secretion of acid by the stomach insignificant in the present experiments.

The concentrations of acids in the meals and recoveries were determined by titration to pH 6·5 against 0·5 N sodium hydroxide using a glass electrode.

RESULTS

First series

Figure 1 shows the volumes of original meal of hydrochloric acid or of citric acid recovered after 20 min plotted against the concentrations of acid in the test meals for H.T. The relationship between concentration and volume recovered appears to be linear for both acids, and this is typical of the results for all acids in all subjects. From Fig. 1 it may be seen that the concentration of hydrochloric acid which gave a recovery of 450 ml. was approximately 45 m-equiv/l. A concentration of 120 m-equiv/l. citric acid gave the same volume of recovered meal. Thus hydrochloric appeared to be nearly three times as effective as citric acid in slowing gastric emptying in this subject.

Regression lines were fitted by the method of least squares to the results for each subject for each acid giving $y = a + bx$, where x = concentration of acid (m-equiv/l.) in the meal, and y = volume of meal recovered (ml.). Table 1 is a summary of the results of 1430 tests in fourteen subjects, with hydrochloric, acetic, lactic, tartaric, citric and phosphoric acids. The value for a represents the volume of meal calculated to be recovered at zero concentration of acid. The value for b is in terms of ml. increase in volume of meal recovered per m-equiv increase of acids per litre of meal. Inspection shows that in general the b values are less for citric acid and tartaric acid than they are for hydrochloric acid and acetic acid.

The regression lines for hydrochloric acid and for citric acid in Fig. 1 cross before they intersect the ordinate at zero concentration of acid, that is plain water. Thus at low concentrations solutions of hydrochloric acid appear to leave the stomach more rapidly than solutions with the same concentration of citric acid. Besides the measurements represented by the points shown in Fig. 1, there are measurements for the volume recovered with water. The mean volume of a water meal recovered, determined experimentally, can thus be compared with the value indirectly obtained

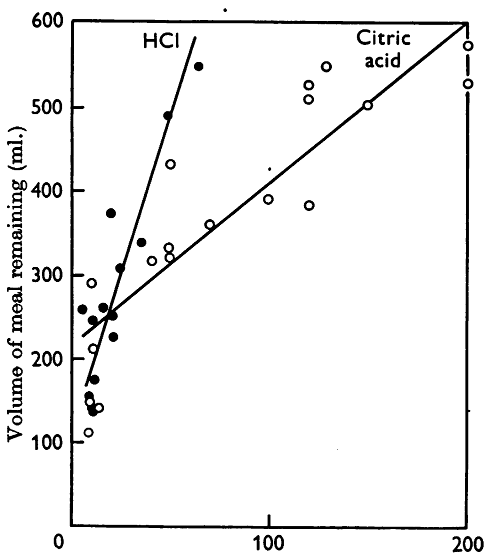


Fig. 1. Slowing of gastric emptying by hydrochloric acid and citric acid in test meals. Subject H.T. Ordinate: volume of original meal recovered 20 min after instillation. Abscissa: concentration of acid in given meal (m-equiv/l.). ● Hydrochloric acid, ○ citric acid.

by extrapolation of the regression lines to zero concentration of acid. The points for water were not included in the calculation of the regression equations. The differences between the direct and indirect measurements of volume of meal recovered are shown in Table 2. In thirteen out of fourteen instances the volume of water recovered was larger than would be expected from extrapolation of the regression lines for concentration of hydrochloric acid plotted against volume ($P = 0{\cdot}005$ using Student's t test).

By the extrapolation of the regression lines for meals of lactic acid the calculated volumes of water recovered were less than those actually recovered in seven out of nine subjects ($P = 0{\cdot}05$). For the other acids such comparisons yielded insignificant differences. The results obtained in this way are consistent with the view that solutions containing low con-

TABLE 1. The effects of acid in test meals on the volume of meal recovered from the stomach after a fixed interval. First series

Volume of meal remaining (y ml.) plotted against initial concentration of acid in the meal titrated to pH 6·5 (x m-equiv/l.) $y = a + bx$ where a corresponds to the volume calculated to be recovered with water, that is the intercept of the regression line and the ordinate in Fig. 1.

n (range) = number of experiments (range of concentrations of acid).

Duration of meals (min)		H.T. 20	F.L. 10	W.S. 10	M.N. 15	S.W. 15	S.T. 20	M.D. 15	R.D. 20	D.M. 20	T.P. 15	C.N. 20	G.D. 30	H.Y. 20	B.R. 20
Hydrochloric	a	128	230	193	250	224	163	122	193	40	269	120	239	233	212
	b	7·09	8·17	12·81	8·14	9·93	4·03	7·79	8·61	10·14	5·82	8·09	2·95	5·20	5·43
	S.E.	0·92	1·40	1·56	1·78	2·18	1·03	1·03	1·72	0·94	1·07	2·03	0·91	1·81	4·47
	n	15	27	13	17	13	22	27	34	18	29	15	37	15	5
	Range	(5–65)	(5–35)	(5–25)	(5–30)	(5–30)	(2–40)	(2–40)	(4–34)	(2–35)	(2–40)	(5–34)	(2–40)	(2–30)	(10–34)
Acetic	a	240	244	245	315	299	149	162	170	73	344	197	225	256	211
	b	5·15	8·05	6·82	5·49	4·41	3·14	3·50	4·10	6·24	3·10	4·56	3·45	4·65	6·76
	S.E.	0·84	1·82	1·79	2·38	0·84	0·67	0·90	0·57	1·09	1·04	0·61	0·79	1·07	2·03
	n	26	19	22	10	10	18	23	24	21	21	13	32	14	7
	Range	(5–65)	(5–30)	(5–30)	(5–30)	(5–30)	(5–57)	(3–57)	(3–57)	(3–50)	(3–48)	(6–48)	(3–57)	(5–48)	(10–40)
Lactic	a	155	—	—	—	—	132	142	174	96	218	94	219	234	—
	b	3·77	—	—	—	—	4·17	4·15	5·92	4·56	4·40	6·81	4·14	4·79	—
	S.E.	0·60	—	—	—	—	1·37	0·95	0·90	1·24	0·72	1·20	0·78	0·90	—
	n	13	—	—	—	—	18	22	29	19	25	13	26	13	—
	Range	(5–79)	—	—	—	—	(5–53)	(2–53)	(2–53)	(8–60)	(2–52)	(5–46)	(2–60)	(5–53)	—
Tartaric	a	119	217	260	260	208	189	103	210	128	307	232	297	238	211
	b	4·82	4·75	3·24	3·50	5·14	1·75	3·76	3·36	2·78	1·99	2·52	1·86	4·10	3·54
	S.E.	0·84	0·88	0·89	1·10	0·62	0·61	0·47	0·53	0·61	0·68	0·67	0·38	0·63	2·16
	n	14	20	20	18	17	14	18	22	19	19	14	24	15	5
	Range	(10–60)	(10–51)	(9–62)	(10–51)	(9–51)	(7–70)	(5–96)	(6–96)	(6–96)	(6–94)	(7–94)	(6–117)	(7–96)	(20–60)
Citric	a	222	281	235	242	257	129	177	183	114	278	148	222	308	322
	b	2·00	2·81	4·39	5·35	3·79	2·07	1·75	2·53	2·41	1·91	2·97	1·87	1·97	1·54
	S.E.	0·27	1·14	0·97	1·25	1·30	0·36	0·35	0·28	0·28	0·48	0·47	0·32	0·60	0·63
	n	17	16	16	11	7	19	19	21	20	19	14	25	12	8
	Range	(10–200)	(5–50)	(5–50)	(5–50)	(5–50)	(18–124)	(20–149)	(13–149)	(10–122)	(13–149)	(18–122)	(13–145)	(13–124)	(21–122)
Phosphoric	a	161	—	—	—	—	152	117	145	133	234	—	226	—	—
	b	5·16	—	—	—	—	4·67	5·02	6·76	3·80	5·25	—	3·12	—	—
	S.E.	0·48	—	—	—	—	0·84	0·95	0·93	1·65	0·96	—	0·74	—	—
	n	22	—	—	—	—	13	32	19	13	17	—	40	—	—
	Range	(8–87)	—	—	—	—	(5–50)	(5–95)	(5–74)	(9–71)	(5–60)	—	(5–95)	—	—
Water volume remaining	Mean	202	247	254	284	230	138	135	218	118	249	176	234	257	253
	S.E.	16·7	12·1	42·8	18·7	41·2	21·8	12·4	12·7	26·6	31·1	13·3	20·0	29·8	37·0
	n	6	7	5	5	3	6	10	9	7	7	4	12	3	2

TABLE 2. Differences between the mean volume of a water meal remaining after a fixed interval and the volume retained calculated by extrapolation of the regression of volume of meal remaining on concentration of acid in the meal (see Fig. 1)

Subjects	H.T.	F.L.	M.N.	W.S.	S.W.	C.N.	S.T.	M.D.	R.D.	D.M.	T.P.	G.D.	H.M.	B.R.	Mean	S.E. of mean
Water–hydrochloric	74	17	55	5	8	56	−25	13	25	78	30	46	24	41	+32	±7·60
Water–acetic	−38	3	39	−60	−69	−25	−9	−27	46	45	−35	22	1	42	−4·6	±10·50
Water–lactic	47	—	—	—	—	82	−32	−7	44	7	87	26	23	—	+31	±13·04
Water–tartaric	83	30	24	0	22	−56	−51	32	7	−10	7	−32	19	42	+8	±9·94
Water–citric	−20	−34	49	13	−27	28	9	−42	35	4	43	30	16	−69	+2·5	±9·49
Water–phosphoric	41	—	—	—	—	—	−14	18	73	−15	19	−10	—	—	+16	±12·37

centrations of hydrochloric acid or of lactic acid leave the stomach more rapidly than plain water. This conclusion would be reaffirmed if it were found by direct measurement that the solutions containing the lowest concentrations of hydrochloric acid or lactic acid, mostly 5 m-equiv/l., left the stomach more rapidly than water. However with the number of measurements available and the considerable variability of the results with low concentrations of acid this point could not be established.

Second series

The first series of experiments contained only one fatty acid, acetic. Because the oil/water partition coefficient was relevant to the interpretation of the results, a series of 586 tests in eight subjects was made with acetic, propionic, butyric, and hexanoic acids. Longer chain acids were not sufficiently soluble in water to give effective concentrations. The results of these experiments are set out in Table 3; this shows the regression coefficients for the relation between volume of test meal recovered after a fixed time and the concentration of acid in the given meal. In every instance increase in the concentration of acid caused an increase in the volume of meal recovered. However, the relative effectiveness of the different acids cannot be judged from the values of b in Table 3 because the intercepts of the regression lines with the ordinate at zero concentration of acid, the value of a, vary considerably within-subject. When the concentrations giving recoveries of 450 ml. of meal were calculated it was found that in general the longer chain acids were less effective, in slowing gastric emptying, in terms of m-equiv/l., than acetic acid. This will be considered more fully below.

DISCUSSION

Comparisons of the effects of different acids. The main object of the present experiments was to compare the effectiveness of different acids in slowing gastric emptying. Such comparisons are more discriminative when there is opportunity for a wide range of variation in slowing. Thus the duration of the test was fixed to give a recovery of about 200 ml. with water, so leaving ample scope for slowing on the addition of acid. S.T. and M.D. were nauseated by water meals of 750 ml. and these two small women were given 600 ml. Since the volumes used and the duration of test varied from subject to subject, the comparisons are relevant within-subject. On the other hand the deliberate variation of the experimental conditions was aimed at standardizing the intragastric conditions between-subjects. Since the results of within-subject analysis are in agreement with the pooled results, they have been used as a convenient summary.

Indices of effectiveness of acids in slowing gastric emptying. The experi-

TABLE 3. The effects of fatty acids in test meals on the volume of meals recovered after a fixed interval. Second series

Volume of meal remaining (y ml.) plotted against initial concentration of acid in the meal titrated to pH 6·5 (x m-equiv/l.). $y = a+bx$ where a corresponds to the volume calculated to be recovered with water, that is the intercept of the regression line and the ordinate in Fig. 1. No. = number of experiments. Range = range of concentrations of acid

		H.T.	G.D.	R.S.	C.L.	M.E.	K.G.	R.U.	R.O.
Hydrochloric	a	128	239	—	197	—	91	160	—
	b	7·09	2·95	—	7·70	—	5·75	7·50	—
	S.E. of b	0·92	0·91	—	1·08	—	1·12	0·98	—
	No.	15	37	—	38	—	27	34	—
	Range	(5–65)	(2–40)	—	(0–30)	—	(0–30)	(0–30)	—
Acetic	a	240	225	172	210	—	76	210	135
	b	5·15	3·45	4·53	5·83	—	4·06	4·86	4·64
	S.E. of b	0·84	0·79	1·88	0·70	—	1·13	0·52	0·63
	No.	26	32	16	18	—	16	24	21
	Range	(5–65)	(3–57)	(0–49)	(0–60)	—	(0–60)	(0–61)	(0–82)
Propionic	a	239	152	224	231	219	169	216	212
	b	2·35	4·82	7·61	6·56	5·51	6·29	4·81	7·92
	S.E.	0·44	0·53	1·28	1·71	0·81	0·94	0·80	1·42
	No.	21	23	26	12	23	12	21	19
	Range	(0–109)	(0–67)	(0–66)	(0–54)	(0–77)	(0·55)	(0–55)	(0–55)
Butyric	a	150	151	165	—	—	88	—	—
	b	3·51	4·94	4·71	—	—	4·58	—	—
	S.E.	0·69	0·51	1·03	—	—	0·87	—	—
	No.	18	16	31	—	—	15	—	—
	Range	(0–60)	(0–75)	(0–71)	—	—	(0–73)	—	—
Hexanoic	a	136	168	184	192	178	76	207	135
	b	3·70	3·30	5·97	4·63	4·69	2·29	3·90	7·04
	S.E.	0·67	0·69	2·05	0·87	0·78	1·75	1·31	0·87
	No.	17	28	21	17	18	14	23	17
	Range	(0–56)	(0–75)	(0–49)	(0–31)	(0–54)	(0–36)	(0–31)	(0–31)

ments consisted of varying the concentrations of acids in the meals and measuring the volume of the meal recovered after a fixed interval. The slopes of the regression lines in Fig. 1, the values of b in Tables 1 and 3, indicate ml. increase in volume recovered per m-equiv increase of acid/l. of given meal. These slopes might be a direct index of the effectiveness of an acid in slowing gastric emptying if the extrapolation of the regression lines for different acids cut the ordinate at the same value. Table 2 shows that this simplification is not valid for comparisons of hydrochloric and lactic acids with the others, which makes this index of little use.

As an alternative the concentration of acid giving a recovery of 450 ml. of meal has been used. This, of course, gives some influence to the value of a in the regression equations of Table 1 and 3. The value of 450 ml. was chosen because it was the largest volume recovered common to all subjects. Substituting 500 or 400 ml. for 450 ml. makes little difference to the index of the effectiveness of the acids.

Two other ways of comparing the action of the acids have been considered. From inspection of Fig. 1 it will be clear that at low concentrations of acid the volume recovered is small so that the volume passing into the duodenum is large, but the amount of acid going into the duodenum, where the relevant receptors are believed to lie, is small. Similarly, at high concentrations of acid the amount of acid going into the duodenum is small because the volume leaving the stomach is small. Between these two extremes there is a concentration of acid which gives a maximal delivery, in terms of m-equiv per min, into the duodenum. This concentration has been computed and used to compare the effectiveness of the different acids. When this concentration is exceeded decreasing amounts of acid are producing progressively more slowing with the implication that the receptor is responding to concentration of acid.

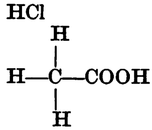
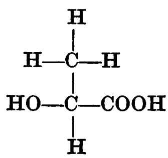
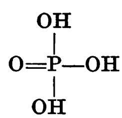
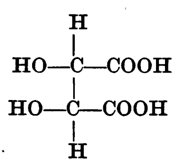
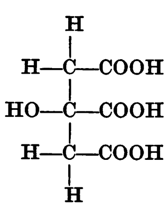
It is not certain that the duodenal receptors are responding only to the concentrations of acid. It is possible that the amount of acid entering the duodenum may also be relevant. Having calculated the concentration giving a maximal delivery of acid to the duodenum, the product of this and the volume leaving the stomach with this concentration gives the maximal amounts of acid leaving the stomach with the different acids. These amounts have also been used for comparative purposes.

The four indices of effectiveness noted above are all closely related to each other by the arithmetic of their derivation. They all have their merits but the concentration giving a recovery of 450 ml. is probably most easily interpreted since the intragastric volumes are the same at the moment of measurement with the different acids. Thus the question of the form of the relationship between receptor activity and the slowing of gastric emptying is avoided.

The properties of acids and the slowing of gastric emptying

The relationship between the molecular weight of an acid and its effectiveness in slowing gastric emptying. The structural formulae of the acids used in Series 1 arranged in order of their effectiveness, their pK values and the calculated mean concentrations of acids in m-equiv/l., giving a recovery

TABLE 4. The structural formulae, pK values and the mean concentrations of acids (m-equiv/l.) giving recoveries of 450 ml. of instilled meal in fourteen subjects

Spatial formula	pK	Mol. wt.	Mean concentration giving 450 ml. recovery
HCl	—	36·5	39
	Acetic 4·8	60	50
	Lactic 3·9	90	62
	Phosphoric 2·1 7·2 12·7	98	61
	Tartaric 3·0 4·3	150	76
	Citric 3·1 4·7 5·4	192	96

of 450 ml. are shown in Table 4. From inspection of the results it became apparent that the greater were the molecular weights of the acids studied, the less effective they were in slowing gastric emptying. To put this judgement on a quantitative basis the concentrations of acids (m-equiv/l.) giving a recovery of 450 ml. were calculated from the results of Table 1 for each

TABLE 5. Regression equations for the within-subject relationship between concentration of acids giving a recovery of 450 ml. after a fixed interval, and the molecular weight of the acids. $y = a+bx$, where x = molecular weight of acids and y = concentration of acids (m-equiv/l.), n = number of acids used, P = probability

	H.T.	F.L.	W.S.	M.N.	S.W.	S.T.	M.D.	R.D.	D.M.	T.P.	C.N.	G.D.	H.Y.	B.R.
a	24·7	15·3	16·0	19·4	20·2	40·2	23·2	20·3	20·5	12·3	26·1	43·8	31·1	26·2
b	0·406	0·228	0·213	0·146	0·168	0·589	0·596	0·391	0·629	0·394	0·390	0·328	0·182	0·284
S.E. of b	0·115	0·027	0·077	0·089	0·033	0·189	0·153	0·125	0·019	0·046	0·049	0·119	0·045	0·061
n	6	4	4	4	4	6	6	6	6	6	5	6	4	4
t	3·53	8·50	2·75	1·64	5·16	3·12	3·90	3·12	33·82	8·51	8·03	2·77	4·02	4·68
P	0·05	0·02	0·2	0·3	0·05	0·05	0·02	0·05	0·001	0·01	0·01	0·05	0·1	0·05

subject and plotted against the molecular weights of the respective acids. Regression lines were fitted and the significance of the regressions assessed by dividing the slopes by their standard errors to give Student's *t*. The regression coefficients are given in Table 5. Individual plots are shown in Fig. 2 in order of their closeness of fit: D.M. 1st, T.P. 4th, M.D. 7th, S.T. 10th.

The fit of the lines in Fig. 2 is good enough to establish the general proposition but must at the same time raise the question as to whether any

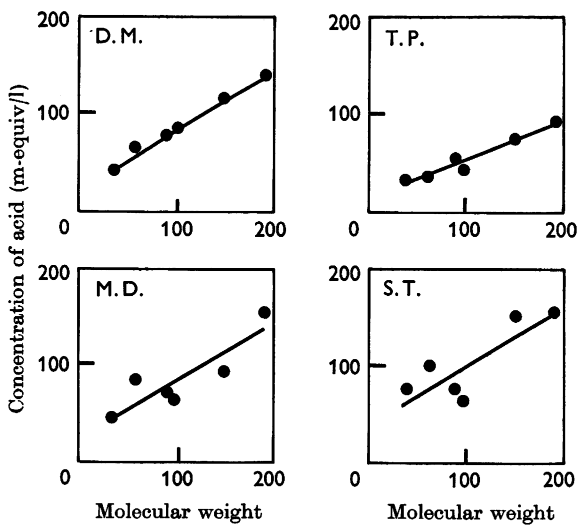


Fig. 2. Relation between the effectiveness of different acids in slowing gastric emptying and the molecular weight of the acid in four subjects. Ordinate: concentraction of acid giving a recovery of 450 ml. after a fixed interval. Abscissa: molecular weight of the acid.

other index of the effectiveness of acid would fit more closely to some function of molecular weight. Table 6 shows the results of an exploration of this possibility by ranking for individuals the values of the slopes divided by their standard errors, that is Student's *t*, for a selection of the possible regressions. The means of these ranks for *t* are statistically indistinguishable when either the concentrations giving 450 ml. recovery or the maximal amounts of acid entering the duodenum per minute are plotted against molecular weight. Figures 3 and 4 show the pooled results plotted in this way. The very close fit in both instances shows that molecular weight is closely linked with effectiveness for these six acids.

When the results were first examined it appeared that effectiveness was more closely linked to the square root of the molecular weights of the acids. At this time Graham's Law seemed relevant and the difference in effectiveness was ascribed to the intervention of diffusion at some stage of the interaction of acid and receptor. The new analysis makes such a notion less attractive but does not rule it out.

The unimportance of the strength and number of replaceable hydrogens in the effectiveness of the acids in slowing emptying. If Figs. 3 and 4 are considered in relation to the results of Table 4 some rather unexpected conclusions may be drawn. The very close fit of the lines to the points indicates

TABLE 6. Mean ranks for Student's *t*. For each subject a linear regression was calculated between the listed variables. For each regression a Student's *t* was obtained by dividing the slope of the regression by its standard error. These *t* values were ranked; the largest was given the lowest rank number. To pool the results for all subjects the mean of the rank numbers for each of the seven types of regressions was calculated. The standard error of this mean rank is also given

	Mean rank	S.E.
Concentrations of acids giving recoveries of 450 ml. plotted against mol. wt.	2·50	±0·51
Maximal amounts of acids entering the duodenum per min plotted against mol. wt.	3·64	±0·63
Concentrations of acids giving recoveries of 450 ml. plotted against $\sqrt{}$(mol. wt.)	4·07	±0·44
Log. of concentrations of acids giving recoveries of 450 ml. plotted against log. mol. wt.	4·71	±0·73
Log. of maximal amounts of acids entering the duodenum plotted against log. mol. wt.	5·57	±0·73
Log. of concentrations giving maximal deliveries to the duodenum plotted against log. mol. wt.	6·71	±0·65
Concentrations of acids giving maximal deliveries to the duodenum plotted against $\sqrt{}$(mol. wt.)	7·00	±0·41

that molecular weight determines the effectiveness of the acid. Since the points for a strong acid such as hydrochloric and for a very weak acid such as acetic fit equally well it seems that the dissociation constant of the acid is irrelevant to its action. The acids used contained one, two or three replaceable hydrogens, but when the results are expressed as m-equiv/l. giving recoveries of 450 ml., this feature is apparently irrelevant to effectiveness. Acetic acid is known to penetrate lipid membranes, both by virtue of its low degree of dissociation and by virtue of its fat solubility. (Teorell, 1939). Yet it seems to have no advantage over citric acid, apart from that conferred by low molecular weight.

The effect of phosphoric acid. Here it is necessary to refer to the end-point of the titrations of acid used in this study. From our previous work (Hunt & Knox, 1962) it was concluded that the duodenal mechanism controlling gastric emptying in effect allowed constant amounts of acid, titratable to pH 6·0, into the duodenum independent of the pH of the gastric contents. This was varied in the experiments by using either citric acid or its monohydrogen or dihydrogen sodium salts. It was implied in the paper that the duodenum and pancreas together acted in such a way as to titrate the citric acid or its sodium salts to pH 6·0. The present results are also

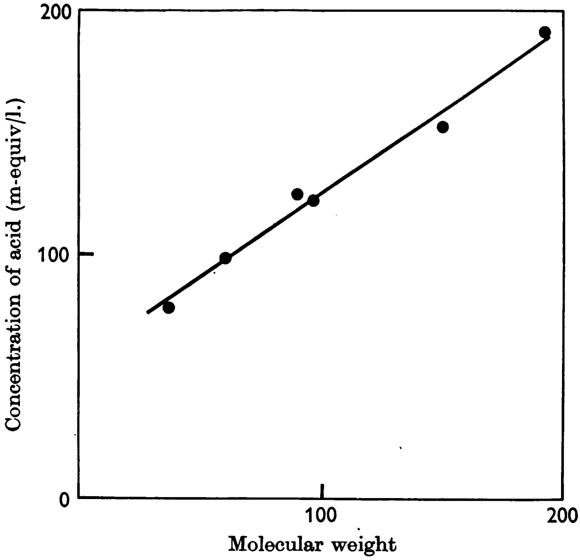


Fig. 3. Relationship between the effectiveness of different acids in slowing gastric emptying and the molecular weight of the acid. Ordinate: mean concentration of acid giving a recovery of 450 ml. after a fixed interval for fourteen subjects. Abscissa: molecular weight of the acid.

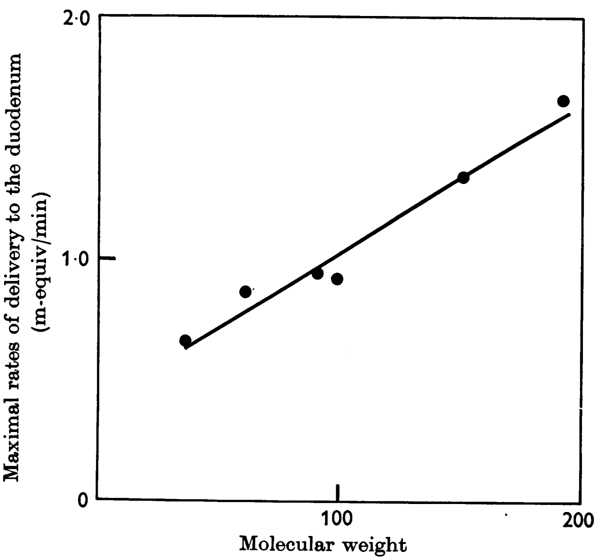


Fig. 4. Relationship between the effectiveness of different acids in slowing gastric emptying and the molecular weight of the acid. Ordinate: mean amounts (m-equiv/min titrated to pH 6·5) of different acids entering the duodenum during a fixed interval when the concentration is adjusted to make the amount maximal for fourteen subjects. Abscissa: molecular weight of the acid.

consistent with the previous view that the intragastric pH has little or nothing to do with the effectiveness of an acid in slowing gastric emptying, since the strong hydrochloric acid and the weak acetic acid fall in their expected place according to their molecular weight in Figs. 3 and 4.

A consideration of the relative effectiveness of hydrochloric acid and phosphoric acid allows the end-point to which the postulated titration occurs to be specified more precisely than has been possible before.

Whether hydrochloric acid is titrated to pH 5·0 or to pH 8·0 makes practically no difference to the resultant calculated concentration expressed in m-equiv/l. This acid dissociates so strongly that over 99 % of the titration is complete at pH 5·0. Thus variation of end-point would not change the value in m-equiv/l. given in Table 4 as causing a recovery of 450 ml.

The situation with phosphoric acid is quite different. It can be determined by titration that at pH 6·5 only 45 % of the titration is complete. Lowering the end-point will lower this value and raising the end-point will raise the titration value expressed in m-equiv/l., correspondingly changing the value of 61 m-equiv/l. given in Table 4. However, the value determined by titration to pH 6·5 gives phosphoric acid the effectiveness that would be expected from its molecular weight, as determined for the other five acids. It may tentatively be concluded that the mechanism slowing gastric emptying in response to acids does work as though it titrates to about pH 6·5.

Further tests of the rectilinear relation between the concentration of acid giving recoveries of 450 *ml. and molecular weight.* It was concluded above that the concentration of an acid in m-equiv/l. which gave a recovery of 450 ml. was independent of the number of replaceable hydrogens in the molecule. However, hydrochloric, acetic and lactic acids with one replaceable hydrogen, fall at one end of the line while tartaric with two replaceable hydrogens and citric with three, fall at the other end of the line which was fitted to all the acids. The regression line is thus bound to fit fairly closely to the points for tartaric and citric acids, since there are no reference points close to them for acids with one replaceable hydrogen.

To provide a more rigorous test of the view that the number of replaceable hydrogens is not material, a regression equation was derived for the points for hydrochloric, acetic and lactic acids with molecular weight as independent variable and concentration of acid as dependent variable for the nine subjects who took all five acids. From this regression the concentrations of acids with mol. wt. 150 (tartaric) and 192 (citric) required to give a recovery of 450 ml. were found to be 82 and 94 m-equiv/l. Experimentally the values were 88 and 117 m-equiv/l. neither of which is significantly different from the computed values.

It appeared possible that the regression lines fitted to concentration of acid giving 450 ml. recovery against mol. wt. in individual subjects might be systematically a poor fit to the results for one or two acids. For example, acetic acid might be expected to be more effective than polar acids because acetic acid would penetrate lipid membranes more readily. If this were so, the point for concentration of acetic acid giving a recovery of 450 ml. would fall consistently below the fitted lines shown in Fig. 2. However, it was found that the points for each acid were randomly disposed about the regression lines for each subject. In effect this means that no systematic 'corrections' of mol. wt. for each acid would improve the correlation between concentration of acid giving a recovery of 450 ml. and mol. wt. At the moment we must conclude that power functions such as mol. wt.$^{\frac{1}{3}}$ or mol. wt.$^{\frac{1}{2}}$ would give a less good fit to the concentration giving recoveries of 450 ml. than the simple molecular weight. This rather unexpected conclusion was examined further by fitting the logarithmic regressions referred to in Table 6. The correlations were significantly less than those for simple molecular weight.

A further test of the irrelevance of the number of replaceable hydrogens per molecule of acid. In a previous paper (Hunt & Knox, 1962) there are results for the relations between the volume of test meal recovered and its concentration of citric acid, monosodium dihydrogen citric acid, and disodium monohydrogen citric acid. If one anomalous result for J.S. is discarded, in four other subjects the mean concentrations in m-equiv/l., giving 450 ml. recoveries are citric acid 77, monosodium dihydrogen citrate 86, and disodium monohydrogen citrate 89. The approximate equality of these values indicates that the number of replaceable hydrogens in the acid is irrelevant in operating the receptor.

The unimportance of oil/water partition coefficient in the effectiveness of acids in slowing gastric emptying. In the first series of acids, acetic was the only fatty acid. In spite of its solubility in lipids and its high pK and its corresponding ability to penetrate lipid membranes, its effectiveness was proportional to its molecular weight. However, it seemed desirable to confirm this point with other acids with greater oil/water partition coefficients. The results for acetic, propionic, butyric, and hexanoic acids were less regular than those for the acids shown in Table 1. However, the greater the molecular weight of the acids the greater was the concentration required to give a recovery of 450 ml. ($P < 0{\cdot}05$).

If the receptor detecting acid lay inside a cell with a lipid membrane it would be expected that the fatty acids would be specially effective in slowing gastric emptying since they would penetrate the membrane more readily than non-fatty acids. Hexanoic acid has a benzene/water partition coefficient of 38 as compared with acetic acid 0·02 (Brown & Bury, 1923).

Figure 5 allows the expectation of increased effectiveness of fatty acids to be tested by comparing the pooled results of Series I with those of Series II. It can be seen that the points for the subjects of Series II fall above those for the subjects of Series I, including the results for hydrochloric acid. Thus the expectation that the fatty acids would be more effective is not borne out by this comparison. The results for subjects H.T. and G.D. which are common to Tables 1 and 3 allow a more rigorous test to be made.

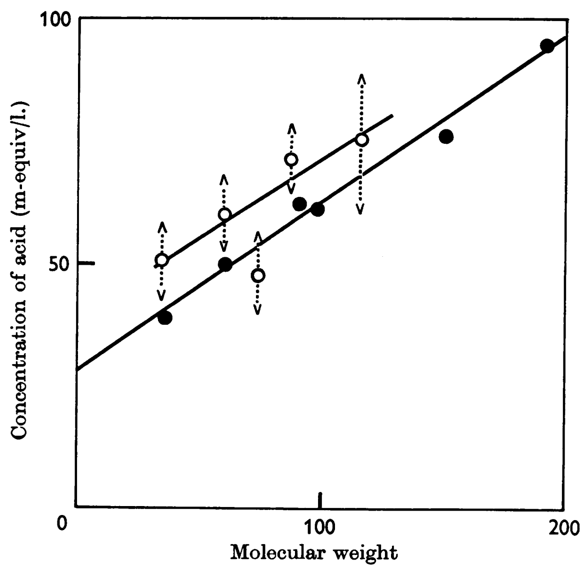


Fig. 5. Comparison of the effectiveness of fatty acids and non-fatty acids on gastric emptying in relationship to molecular weight. Ordinate: mean concentrations giving 450 ml. recoveries of meal after fixed interval. Abscissa: molecular weight of acid. ● Series I in fourteen subjects mainly non-fatty acids, ○ Series II in eight subjects mainly fatty acids.

Regressions of the concentration of acids giving a recovery of 450 ml. and the mol. wt. of the acid were computed for hydrochloric, lactic, tartaric, citric and phosphoric acid, and for acetic, propionic, butyric and hexanoic acids for H.T. and for G.D. The regressions were then recomputed pooling the results for hydrochloric etc. with acetic etc. The residual means squares for H.T. were, Series I, 263, Series II, 1037, and pooled, 322: for G.D., Series I, 309, Series II, 77, and pooled, 157. Since the residual mean squares for the pooled results were less than the larger of the residual mean squares for Series I and Series II, there is no evidence to suggest that the points for fatty acids come from populations different from the points for non-fatty acids. This is more clearly brought out in Fig. 6 which shows the means for the concentrations of acid giving recoveries of 450 ml. in H.T. and G.D. plotted against the mol. wt. of the acids. There is no indication that the points for fatty acids fall below the regression line as they would if the fatty acids were more effective than the non-fatty acids.

Increase in the rate of gastric emptying of acids. Hedblom & Cannon (1909) established that low concentrations of hydrochloric acid increased the rate of gastric emptying of potato in cats whereas high concentrations slowed it relative to potato without acid.

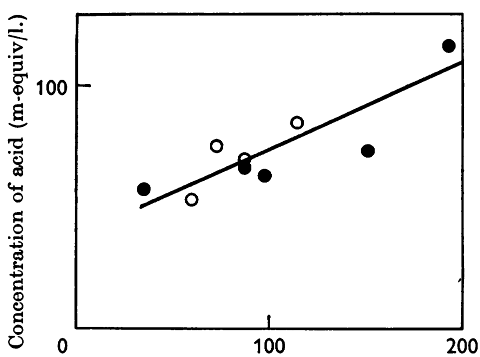


Fig. 6. Comparison of the effectiveness of fatty and non-fatty acids in relation to molecular weight in H.T. and G.D. Ordinate: mean concentration giving recovery of 450 ml. Abscissa: molecular weight of acid. The regression line was fitted to all the points. ○ Fatty acids, ● non-fatty acids.

The result shown in Table 2, that hydrochloric acid and lactic acid at low concentrations appear to leave the stomach more rapidly than water, confirms the work of Pathak (1959) and is consistent with that of Hedblom & Cannon (1909). However, the results of our experiments are not absolutely convincing since the conclusion depends upon an extrapolation.

Implicatious

The results appear to put some definite constraints on the receptor mechanism by which acids slow gastric emptying.

1. There is no doubt that the receptor system responds to acid since the sodium salts of hydrochloric acid, and of citric acid hasten gastric emptying as compared with the emptying of water (Hunt & Pathak, 1960; Hunt & Knox, 1962).

2. The response varies with m-equiv of acid per litre of meal. The pK of the acid is irrelevant provided it is below about 5. More precisely the system responds to about 45 % of the replaceable hydrogen of phosphoric acid. It can be supposed that the receptor system in effect titrates aliquots

of the acid meals to pH 6·5 and that the response is proportional to the amount of base required. There is no other way to measure the concentration of various acids in m-equiv/l. without knowledge of their pK values. A biological system which measures pK is conceivable, but this notion will be discarded because it is more complex than the titrator device above.

3. The provision of a base involved in the titration of the acid, for example bicarbonate ion, can be imagined to leave behind hydrogen ion at the detector site. Such a system can be considered to give all acids in test meals the same pK at the detector site. A detector of the type postulated for the respiratory response to H_3O^+ or CO_2 could then give a signal in proportion to the base lost into the acid of the test meal as it traversed the duodenum. It can be assumed that the rate at which the generation of base occurs is governed by the depth of the acid sink in the duodenum. This would be analogous to the system generating ammonia in the kidney in proportion to the pH of the urine.

4. It remains to suggest a mechanism to account for the way in which increase in molecular weight of the anion reduces the effectiveness of each m-equiv of acid. It is reasonable to suppose that acids of high mol. wt. either undissociated or in the anionic form paired with H_3O^+ would diffuse from the duodenal lumen into the surrounding tissue more slowly than those with low molecular weights. If titration occurred in the duodenal lumen, acids with high molecular weights would be more effective than those with low, because they would not diffuse into the tissues (Winship & Schultz, 1967). The decreased effectiveness of acids with high molecular weights is therefore consistent with the postulated titration occurring at a site deep to an aqueous diffusion barrier or filter such as the intestinal mucous layer which retards the access of the acids with high molecular weights to the site of the titration.

5. Acetic acid and the other fatty acids are largely undissociated in an aqueous solution, a fact which is used to explain their relatively rapid penetration of lipid membranes (Teorell, 1939). The uncharged acid is considered to penetrate by virtue of its solubility in lipids. However, these properties have not significantly disturbed the effectiveness of the fatty acids of Series II in slowing gastric emptying which is more or less as expected from their molecular weight. The need to assume titration of acids based on the reasoning above and the unexceptional action of fatty acids are consistent with the receptor system for slowing gastric emptying working predominantly in an aqueous phase.

The relationship between effectiveness and molecular weight was discovered by comparing the concentrations of acids required to give equal slowing of gastric emptying. Under these conditions it can be assumed that the whole receptor effector system is equally active with the different acids. The molecular weight/effectiveness relationship is presumably determined by prereceptor events, possibly by molecular sieving.

The tentative explanation of the results put forward here has not concerned itself so far with the realities of events within the duodenum (Lagerlof, Rudewald & Perman, 1960). Presumably the acid of the meal stimulates the release of secretin which activates the pancreas, thus neutralizing the acid. The secretin might itself be one mediator of the slowing of gastric emptying (Chey, Hitanant, Hendricks & Lorber, 1968). If so, the properties discussed above might be those of one of the receptors releasing secretin.

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REFERENCES

BLOOM, D. S., JACOBSON, E. D. & GROSSMAN, M. I. (1967). Validation of dilution indicators in the stomach. *Gastroenterology* **52**, 205–210.

BROWN, F. S. & BURY, C. R. (1923). The distribution of normal fatty acids between water and benzene. *J. chem. Soc.* **123**, 2430–2434.

CHEY, W. Y., HITANANT, S., HENDRICKS, J. & LORBER, S. H. (1968). Effect of intestinal hormones on human gastric function. *Gastroenterology* **54**, 1225.

HEDBLOM, C. A. & CANNON, W. B. (1909). Some conditions affecting the discharge of food from the stomach. *Am. J. med. Sci.* **138**, 504–521.

HIRSCH, A. (1893). Untersuchungen über den Einfluss von Alkali und Säure auf die motorischen Funktionen des Hundemagens. *Zentbl. klin. Med.* **14**, 73–77.

HUNT, J. N. & PATHAK, J. D. (1960). The osmotic effects of some simple molecules and ions in gastric emptying. *J. Physiol.* **154**, 254–269.

HUNT, J. N. & KNOX, M. T. (1962). The regulation of gastric emptying of meals containing citric acid and salts of citric acid. *J. Physiol.* **163**, 34–45.

LAGERLOF, H. O., RUDEWALD, M. B. & PERMAN, G. (1960). The neutralisation process in the duodenum and its influence on gastric emptying in man. *Acta med. scand.* **168**, 269–284.

LORBER, S. H. & SHAY, H. (1956). The duodenal mechanism in the control of gastric motility. *Gastroenterology* **31**, 117–130.

PATHAK, J. D. (1959). Some effects on introducing HCl into human stomach. *Indian J. med. Res.* **47**, 325–328.

SHAY, H. & GERSHON-COHEN, J. (1934). Experimental studies in gastric physiology in man II. A study of pyloric control. Role of acid and alkali. *Surgery, Gynec. Obstet.* **58**, 935–955.

SHAY, H., GERSHON-COHEN, J. & FELS, S. S. (1939). The absorption of hydrochloric acid by the human stomach. *Am. J. dig. Dis.* **6**, 361–363.

TEORELL, T. (1939). On the permeability of the stomach mucosa for acids and some other substances. *J. gen. Physiol.* **23**, 263–274.

WINSHIP, D. H. & SCHULTZ, W. J. (1967). Loss of H^+ in the intact human duodenum. *Gastroenterology* **52**, 1144.