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THE SLOWING OF GASTRIC EMPTYING BY PROTEINS IN TEST MEALS

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SUMMARY

1. Test solutions containing either glucose, casein, partially hydrolysed gelatin or egg albumin, native or denatured, at concentrations up to 50 g/l. in 33 mM-tri-sodium citrate solution, were given by tube into the stomachs of nine subjects in volumes of 600 or 750 ml.

2. The volume of the test solution recovered from the stomach after 20 min was measured.

3. The greater the concentration of solute the greater was the volume of the test solution recovered.

4. Gram for gram, gelatin hydrolysate was equivalent to glucose in slowing gastric emptying. Casein was slightly more effective than glucose and native egg albumin was less effective than glucose.

5. The results were explained by assuming that the products of hydrolysis of proteins stimulated osmoreceptors in the walls of the duodenum.

6. The results were in line with the suggestion that proteins in food contribute to the slowing of gastric emptying in such a way that isocaloric amounts of carbohydrate and mixed protein have the same effect.

7. There was a strong correlation between the concentration of glucose giving a recovery of 400 ml. and the corresponding concentration of casein within-subject, but there was no such relationship for gelatin hydrolysate. It was concluded that the between-subject variability in the digestion of casein was small, that for gelatin hydrolysate was large.

INTRODUCTION

Carbohydrates, fats and amino acids in meals slow gastric emptying (Cooke, 1975); the greater their concentration, the greater is the slowing, measured as ml./min (Hunt & Stubbs, 1975). Although it is to be expected that proteins also will slow gastric emptying, since the products of their digestion instilled into the duodenum of dogs inhibits gastric peristalsis (Thomas & Crider, 1939), quantitative results for man are not known to us. Such information is needed to test a proposal of Hunt & Stubbs (1975) that, in man, isocaloric concentrations of carbohydrate, fat and protein in food would slow gastric emptying equally.

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It is concluded below that some proteins did slow gastric emptying, probably through the action of the products of their hydrolysis on duodenal osmoreceptors. Equal weights of protein or glucose slowed gastric emptying to about the same extent.

METHODS

The test solutions consisted of one of the following in concentrations of 5, 10, 20, 30, 40 or 50 g/l. Glucose monohydrate (mol. wt. 198). Na caseinate (Upjohn, Michigan, U.S.A. contains 0·8 m-mole Na^+/g). Partial hydrolysate of gelatin (Kind and Knox Gelatin, Iowa, U.S.A. mean mol. wt. *ca.* 6000). Egg albumin (Behr, London, S.E.1). Denatured egg albumin (as for the native form, but the solutions were brought to the boil before use).

All the test solutions were made up with 33 mM-Na citrate to keep the proteins in solution. Some test solutions consisted of 33 mM-trisodium citrate with nothing further added. Occasionally a small amount of one of the forms of egg albumin precipitated in the stomach causing blockage of the gastric tube. Consequently results for the two egg albumins were available up to concentrations of only 30 or 40 g/l. for some subjects. The volume of the test solutions was 750 ml. for all subjects, except for two small women, all of whose solutions were of 600 ml., as the large volume caused them discomfort.

The subjects were nine healthy adult volunteers. After overnight fasting they took 250 ml. distilled water at room temperature and recovered as much of the gastric contents as they could. Then a test solution, previously warmed to 37 °C, was instilled into the stomach through a tube in less than 2 min. 20 min after beginning the instillation the gastric contents were recovered and the stomach immediately washed out again with 250 ml. distilled water at room temperature. One meal was taken per day. The experiments were spread over several months in no particular order. All the test solutions contained phenol red (about 60 mg/l.), a dye that is not absorbed from the stomach (Ivey & Schedl, 1970). The volume of the test solutions recovered was assessed from the recovery of phenol red in the gastric contents plus that in the washout (Hunt & Knox, 1962).

Statistical methods

The regressions for the volumes of the test solution recovered from the stomach after 20 min (y ml.) against the concentration of the solute (x g/l.) were calculated by the method of least-squares for each material in every subject. Although the intercepts of the regression lines on the ordinate were not systematically different from the mean volumes recovered with the trisodium citrate alone, these results for zero concentration of solute were not included in the regression lines, as they were common to all materials. The slopes for 'glucose' were corrected to be for glucose, mol.wt. 180, and not for glucose monohydrate, mol.wt. 198, by multiplying by 198/180. This corrected slope was used in calculating the concentrations giving recoveries of 400 ml. (Table 2). The implications of assessing the effectiveness of solutes by this procedure were discussed by Hunt & Knox (1969).

RESULTS

Concentrations of proteins giving recoveries of 400 ml. of test solution

The relations between the volumes of test solution recovered from the stomach after a fixed interval and the concentration of the given solute are shown in the regression equations of Table 1. The slopes of the regression lines and their intercepts were compared by within-subject t tests. No significant differences were found between solutes when attention was paid to the effect of multiple comparisons (Dunnet, 1964). A further comparison of the effectiveness of the various solutes in slowing gastric emptying was made by calculating the concentration of each solute giving a recovery of 400 ml. of the original solution. Based on nine subjects (Table 2) the mean concentrations giving recoveries of 400 ml. were (g/l.) glucose 48·8, casein 45·4 and gelatin hydrolysate 59·3. By paired t tests it was not possible to dismiss the hypothesis that these values came from a population with a common mean.

TABLE 1. Relation between concentrations of proteins and volume of test meal recovered

	Glucose					Na⁺ caseinate					Gelatin hydrolysate					Egg albumin (denatured)					Native egg albumin					Mean citrate recover (ml.)		
	n	*a*	*b*	± S.E.	range g/l.	*n*	*a*	*b*	± S.E.	range g/l.	*n*	*a*	*b*	± S.E.	range g/l.	*n*	*a*	*b*	± S.E.	range g/l.	*n*	*a*	*b*	± S.E.	range g/l.	*n*	mean	*Sl*
B.M.	18	29	6·7	0·9	5–50	25	135	4·9	1·2	10–50	22	90	4·6	1·1	5–50	12	28	7·0	2·0	5–30	14	18	5·9	1·3	5–50	9	72	19
C.W.	13	9	7·8	0·7	5–50	15	135	5·7	1·1	5–50	13	26	6·3	0·9	5–50	13	73	4·9	1·7	5–50	18	86	1·8	1·7	5–50	4	38	15
F.R.	20	226	5·9	2·1	5–50	12	111	8·5	0·7	5–50	16	157	3·5	0·7	5–50	10	113	5·9	1·6	5–50	10	88	4·4	1·1	5–50	4	152	25
H.T.	11	−25	9·4	1·2	5–50	13	5	9·4	1·6	5–50	15	39	5·1	0·8	5–50	10	95	4·5	2·0	5–40	15	106	1·4	1·1	5–50	2	73	55
P.L.	16	113	4·4	1·2	5–50	14	93	5·3	1·2	5–50	13	175	4·1	0·6	5–50	4	203	1·6	1·5	5–30	8	98	2·5	1·8	5–40	5	69	24
W.Y.	6	37	5·7	2·1	10–40	6	204	3·4	1·2	10–50	11	135	6·2	1·3	5–50											4	68	34
G.D.	10	75	5·7	1·0	5–50	12	40	7·3	1·0	5–50	13	70	3·4	1·1	5–50											3	65	33
G.M.	9	145	7·1	1·9	5–50	5	288	3·7	1·3	5–50	6	286	5·0	2·3	5–40											3	133	14
C.N.	8	179	6·0	2·2	5–50	7	223	4·8	0·8	10–50	8	215	4·4	1·2	5–50											3	229	101

Regression equations for volume of test solution recovered after 20 min (y ml.) against the concentrations of proteins or glucose in the given solution (x g/l.). All solutions were made in 33 mM-trisodium citrate. Results for tests with zero concentrations of protein were not included in the regressions. $y = a + bx$, a = intercept (ml.), b = slope (ml. increase volume recovered/g.l.), n = number of tests, ± S.E. = standard error of b, Range = concentration of solutes in test meals.

The mean concentrations (g/l.) giving recoveries of 400 ml. of the original solution in the five subjects who took egg albumin were glucose 49·1, casein 46·9, gelatin hydrolysate 65·9, denatured egg albumin 71·9 and native egg albumin 128·8. Apart from the results for native egg albumin it was not possible on these results to dismiss the notion that these proteins were equally effective in slowing gastric emptying. By analysis of variance it was found that native egg albumin was less effective in slowing gastric emptying than the other proteins tested ($P < 0{\cdot}01$, not allowing for multiple comparisons). This can be confirmed by reference to Table 1, C.W. and H.T. The slowing of gastric emptying by native albumin was significantly less than by glucose ($P < 0{\cdot}01$).

TABLE 2. Concentrations giving recoveries of 400 ml. after 20 min

	Glucose (g/l.)	Na^+ caseinate (g/l.)	Gelatin hydrolysate (g/l.)	Egg albumin denatured (g/l.)	Egg albumin native (g/l.)
B.M.	55·3	54·1	67·4	53·1	64·7
C.W.	50·1	46·5	66·8	66·7	177·7
F.R.	29·8	34·0	69·4	48·6	70·9
H.T.	44·9	42·0	70·8	67·8	210·0
P.L.	65·2	57·9	54·9	123·1	120·8
Mean	49·1	46·9	65·9	71·9	128·8
S.E. ±	5·9	6·1	12·7	13·4	28·8
W.Y.	63·5	57·6	42·7		
G.D.	57·9	49·3	97·1		
G.M.	35·7	30·3	22·8		
C.N.	36·5	36·9	42·0		
Mean of 9	48·8	45·4	59·3		
S.E. ±	4·3	3·4	7·2		

Concentrations (g/l.) of glucose and proteins giving recoveries of 400 ml. test solutions after 20 min. The values were computed from the results of Table 1 which exclude results for zero concentration of solute.

DISCUSSION

Do amounts of carbohydrate and protein having equal available energy slow gastric emptying equally?

It is generally believed that the greater the concentration of fat or carbohydrate in a meal the greater will be the slowing of gastric emptying (ml./min) (Cooke, 1975). It was proposed that the ratio of the effectiveness of carbohydrate and fat in slowing gastric emptying, although in no way caused by their energy, was nevertheless proportional to their content of metabolically available energy, 4 g triglyceride being equivalent to 9 g carbohydrate, each = 36 kcal (150 kJ) (Hunt & Stubbs, 1975). These authors found that rates of gastric emptying predicted from the energy density (kcal/ml.) of mixed food gave a closer match to the measured rates if they assumed that protein also slowed gastric emptying. They assigned an energy content of 4 kcal/g to the protein in predicting rates of gastric emptying from the energy density of the given meal.

The notion that all proteins slow gastric emptying equally can be dismissed on the

basis of the results for native egg albumin which was significantly less effective than glucose in C.W. and H.T. (see Table 1). As will be explained below the effect of protein probably depends on its digestion in the duodenal lumen and in the duodenal mucosa. Native egg albumin has a reputation for being digested with difficulty (Terroine, 1933; Mauron, 1972). Four out of five subjects showed slowing of gastric emptying by denatured (boiled) egg albumin at least equal to that by gelatin hydrolysate. The anomalous results for P.L. are based on only four tests so that they have little intrinsic reliability.

The validity of the notion that mixtures of food proteins will slow gastric emptying equally and that 1 g protein will be equivalent to one gram of glucose can be assessed from the results in Table 2. Leaving out the anomalous results for P.L., the means in general do not disturb the idea that food proteins and carbohydrates might be equally effective gram for gram, and therefore kcal for kcal. However, the present range of proteins tested is an inadequate basis for generalization (we chose gelatin hydrolysate because it was soluble, of low viscosity, specifiable, harmless and inexpensive). However, the quantitative agreement between the means in Table 2 is impressive. Since less than 15 % of the energy in the diet comes from proteins (Ministry of Agriculture Fisheries and Food, 1967) such variability between the effects of various proteins as appears in Table 2 would presumably by of minimal metabolic significance.

A relation between slowing of gastric emptying by glucose, by casein, and by gelatin hydrolysate

It was proposed in the introduction that proteins, after hydrolysis to oligopeptides and amino acids slowed gastric emptying by stimulating duodenal osmoreceptors. The duodenal osmoreceptor is also supposed to be operated by glucose in its native state (Barker, Cochrane, Corbett, Hunt & Kemp Roberts, 1974). Thus differences between subjects in their gastric emptying of a glucose solution should reflect the between-subject variability in the gastric pumping mechanism and in the inhibitory power of the duodenal osmoreceptor. The differences between subjects in response to casein reflects the variability in the gastric and duodenal components relevant to glucose, plus any between-subject variability there may be in the duodenal digestion of casein.

It may be seen that in Fig. 1 the points relating concentration of glucose giving 400 ml. recoveries to the corresponding concentration of casein for different subjects fall closely about a straight line. The implication here is that the digestion of casein is similar in all subjects, since it has added little variance to the responses of the system slowing gastric emptying in response to glucose. Since the tests with glucose and with casein were made on separate days, there was presumably little between-day variability in the components of the system. There is no sign in the standard errors of the slopes in Table 1 that the responses are more variable for casein than for glucose. Since the regression lines shown in Fig. 1 relating concentrations of casein giving recoveries of 400 ml. to those of glucose giving the same recovery has a slope significantly less than 1 ($P < 0{\cdot}05$) it may be concluded that by this test casein is more effective than glucose in slowing gastric emptying.

The remaining points of Fig. 1 are for the relation between the concentrations of

glucose and the concentrations of gelatin hydrolysate giving recoveries of 400 ml. Statistically there is no relation between these two values in each subject ($P > 0{\cdot}5$). The variability of response *within-subject* is no greater for gelatin hydrolysate than it is for glucose.

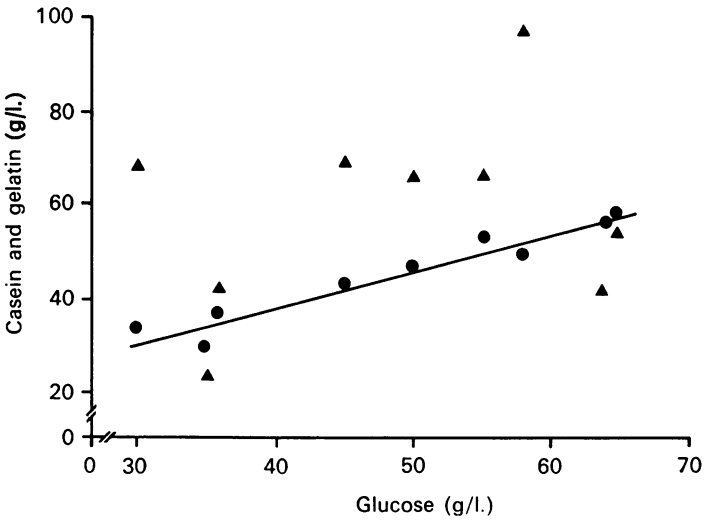

Fig. 1. Concentrations (g/l.) of glucose (x) giving recovery of 400 ml. original solution after 20 min plotted against the corresponding within-subject concentration for casein y (●) and gelatin z (▲) hydrolysate. (Based on Table 2.)

(Casein) $y = 7{\cdot}8 + 0{\cdot}77x$ (glucose). S.E. of slope $\pm 0{\cdot}08$. Residual mean square = 8·7. Line shown in figure.

(Gelatin hydrolysate) $z = 39{\cdot}1 + 0{\cdot}42x$ (glucose). S.E. of slope $\pm 0{\cdot}62$. Residual mean square = 498. Line not shown in Figure. Regressions fitted by least-squares.

Inspection of the results for native egg albumin shows no relation between the concentrations giving recoveries of 400 ml. and the corresponding values either for glucose or for casein. We conclude that the result shown in Fig. 1 can be explained by assuming that in our nine subjects digestion of gelatin varies from person to person, but is consistent within-person. The idea that slow rates of gastric emptying indicate rapid, or thorough, duodenal digestion of protein can be given quantitative form.

An assessment of the mean molecular weight of the products of digestion of protein

If solutions containing equal masses of casein hydrolysis products and of glucose (mol. wt. 180) per unit mass of water, have equal vapour or osmotic pressures, the mean effective mol.wt. of the products of hydrolysis of casein must be 180. If it is assumed that the slowing of gastric emptying is mediated through the osmotic pressure of the products of hydrolysis of casein, when 1 g glucose is equivalent to 1 g casein it may be inferred that the mean molecular weight of the hydrolysis products is 180. This train of thought may be applied to the results of Table 2 to assess the mean effective molecular weights of the products of hydrolysis slowing gastric emptying in the present experiments. It was found that 48·8 g glucose/l.

and 45·4 g casein/l. gave a mean recovery of 400 ml. Thus the weighted mean molecular weight of the hydrolysis products of casein was assessed as $180 \times 45{\cdot}4/48{\cdot}8 = 167$ (133). The value in parentheses is the mean molecular weight of the amino acids in casein (based on the composition given by Ling, Kon & Porter, 1961). The corresponding values were for gelatin hydrolysate 218 (110) (Eastoe & Leach, 1958) denatured egg albumin 264 (134) and native egg albumin 472 (134) (Ling *et al.* 1961). The computed values for the mean effective molecular weights of the products of hydrolysis are all more than the values expected on the basis of complete hydrolysis of the proteins to amino acids ($P < 0{\cdot}01$ by paired t tests within-subjects). This puts some constraints on the mode of action of the osmoreceptor.

The mechanism by which proteins slow gastric emptying

The suggestion here is that proteins slow gastric emptying by the action of the products of their hydrolysis on duodenal osmoreceptors (Hunt & Knox, 1968). It is known that amino acids slow gastric emptying (Cooke & Moulang, 1972; Barker *et al.* 1977). They probably do so through the osmoreceptor mechanism since amino acids, glucose, and potassium chloride solutions of the same osmolal concentration are equally effective in slowing gastric emptying (Barker *et al.* 1974; Cooke & Moulang, 1972).

As the effective molecular weight of the digestion products acting upon the osmoreceptors is above that of their constituent amino acids, could proteins exert their effects through a mixture of amino acids and small oligopeptides? Some oligopeptides can be absorbed into the epithelial cell intact, hydrolysis occurring at the brush border or in the cytosol, with small amounts of oligopeptides passing through to the blood (Matthews & Adibi, 1976). The osmoreceptors lie deep to the brush border because, gram for gram, maltose is almost as effective as glucose (Elias, Gibson, Greenwood, Hunt & Tripp, 1968). According to our hypothesis, maltose must have been acted upon by the disaccharidases, which lie on the brush border (Miller & Crane, 1961), before it reaches the osmoreceptors. It has been proposed that the lateral channels act as the osmotically sensitive component of the osmoreceptors (Barker *et al.* 1977) which could allow their cytoplasmic surface to be exposed to a mixture of amino acids and oligopeptides that are believed to be transported into the enterocyte. However, the evidence is against oligopeptides exerting an osmotic effect from within the cell. Diglycine, believed to be absorbed as such (Matthews & Adibi, 1976) slows gastric emptying as though it were completely hydrolysed to glycine (G. R. Barker *et al.* in preparation). Extrapolating from this single example for lack of any others, it may be postulated that all oligopeptides are virtually completely hydrolysed in the cytosol. If this can be accepted, it limits the interpretation of the computed mean effective molecular weights for the products of hydrolysis. For example, the mean molecular weights of the amino acids of gelatin is 110, but the apparent molecular weight of the products of hydrolysis is, say 220. But oligopeptides, mol. wt. 220, are immediately split into amino acids, mean mol.wt. 110, in the cytosol of the enterocyte. On this argument there is no opportunity for oligopeptides to act from the cytosol of the enterocyte on the osmoreceptive lateral channel. Extending this, one could suppose that half the hydrolysed protein, on a g/l. basis, had entered the cytosol and exerted an osmotic pressure proper to complete hydrolysis to amino

acids, and half had remained in the duodenal lumen with a molecular weight so large, say over 1000, as to exert no detectable osmotic effect.

Since the usual concentration of protein in the gastric contents is likely to be less than 50 g/l., half the protein in a form with molecular weight of 1000 would give an osmolal concentration of only 25 m-osmole/l. which the osmoreceptor would not detect in the presence of 25 g amino acids, mol.wt. 110, giving an osmolal concentration of 230 m-osmole/l.

Possible nutritional implications of the slowing of gastric emptying by proteins

It seems reasonable to suppose that the slowing of gastric emptying by protein depends upon its digestion in the duodenum. Thus it would be expected that patients with severe deficiency of pancreatic enzymes would empty protein solutions from their stomach relatively quickly as compared with their rate of emptying of glucose, corresponding to the situation for starch and triglycerides (Mallinson, 1968; Knox & Mallinson, 1971).

The small between-subject variability in the slowing of gastric emptying by casein relative to the large between-subject variability with gelatin hydrolysate, if extended to other proteins and the more distal regions of the gut, might have nutritional implications when a source of protein in food is changed, say from animal to vegetable protein. From the results of the present experiments it seems quite plausible that meals of mixed proteins and carbohydrates having equal available energy, should slow gastric emptying equally.

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