

## The course of digestion of different food proteins in the rat

### 2\*. The effect of feeding carbohydrate with proteins

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*(Received 15 July 1970—Accepted 2 October 1970)*

1. Single meals of protein (24.3 mg nitrogen/100 g body-weight) were fed with and without carbohydrate (167 mg/100 g body-weight) to groups of rats kept in anticoprophagy cages after an 18 h fast. The contents of the gastro-intestinal tract were collected and analysed and the rises in plasma amino acid concentrations were also determined.

2. After ingestion of different proteins with sucrose, the passage of protein from the stomach was delayed compared with that when the meal was of protein alone: the initial stomach emptying was little affected by the nature of the protein in the diet, but subsequently the relative rates of evacuation of different proteins were similar to those when the proteins were given alone.

3. When proteins were given with different carbohydrates the subsequent digestion and absorption of the meal was modified in a way which could be explained by the observed properties of the carbohydrates given alone, particularly with regard to solubilization in the stomach.

4. The rises in concentration of plasma free amino acid were lower after ingestion of proteins with carbohydrate than when the proteins were eaten alone, and different carbohydrates affected these rises to different degrees.

The response of an animal to a particular protein has been shown to depend upon the carbohydrate in the meal (e.g. Guggenheim, Halevy & Friedmann, 1960). Several workers have observed that the complex carbohydrates were superior to the simple sugars in tests such as protein efficiency ratio and growth rate (Henderson, Deodhar, Krehl & Elvehjem, 1947; Hanks, Henderson, Brickson & Elvehjem, 1948; Harper & Katayama, 1953; Chang, 1962). This has been attributed to a slower passage of the digesta with more complete amino acid liberation (Register & Peterson, 1958) although Chang (1962) found no improved digestibility and Spivey, Katayama, Yoshida & Harper (1958) observed no improved release or absorption of amino acids. A too rapid movement through the intestine may impair not only the digestion of the dietary protein but also that of the endogenous secretions, leading to severe nitrogen losses (Harper, Katayama & Jelinek, 1952).

In the first paper in this series (Zebrowska, 1968) and in work to be reported in a later paper it was found that feeding with different dietary proteins without any supplement resulted in widely differing rates of stomach emptying and levels of nitrogen and dry matter in the gastro-intestinal tract. In the work described here the modifying activity of different carbohydrates on protein digestion was investigated and compared with the behaviour of carbohydrates ingested alone.

\* Paper no. 1: *Br. J. Nutr.* (1968), 22, 483.

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## EXPERIMENTAL

*Materials*

Glucose, sucrose, lactose and soluble starch were obtained from British Drug Houses Ltd, Poole, Dorset and maize starch and maize dextrin from Brown & Polson Ltd, London. The sources of the proteins were as detailed by Zebrowska (1968).

*Tests with rats*

All the meals were given to groups of six or more rats kept in anticoprophagy cages after an 18 h fast. The proteins were given in amounts calculated to supply 24.3 mg nitrogen/100 g body-weight and the carbohydrate, whether alone or with protein, was given at 167 mg/100 g body-weight. At various times after feeding, the animals were anaesthetized and 4 ml samples of blood were taken into heparinized syringes from the portal vein and the heart. The samples were cooled in ice before centrifuging at 1250 g at 1-5°. The plasma was deproteinized with 5 vol. of 3% (w/v) sulphosalicylic acid and the supernatant fraction stored at -20°. The plasma free amino acid levels were determined in pooled samples by an adaptation of the procedures of Moore, Spackman & Stein (1958) and Spackman, Stein & Moore (1958) using an automatic amino acid analyser (Evans Electroelenium Ltd, Halstead, Essex). At the same times, the contents of the stomach and small intestine were collected and the nitrogen and dry-matter contents determined. The pooled soluble gut contents were subsequently analysed by filtration in Sephadex G-25 gel-filtration medium. Details of the experimental procedure were described by Zebrowska (1968).

## RESULTS AND DISCUSSION

*Effect of ingestion of proteins with sucrose*

The nitrogen and dry-matter contents in the stomachs and small intestines of rats 1, 2 and 3 h after feeding on casein and  $\alpha$ -protein with and without sucrose are shown in Table 1.

In the stomach the nitrogen and dry-matter contents were broadly similar after 1 h for each of the proteins used, but they diverged after this time. Since the protein and sucrose meal was a large one for the rat, a possible explanation is that after larger meals stomach evacuation proceeds initially according to mass but that the nature of the diet affects the rats after this time. The most rapid passage of food from the stomach always took place in the immediate postprandial period.

The addition of sucrose to the diet caused a notable reduction in the rate of passage of the protein from the stomach at all times after feeding. The soluble dry matter was increased several-fold in the stomach but not in the small intestine, showing that although sucrose was highly soluble it was rapidly absorbed. The soluble nitrogen level in the stomach was not greatly affected by the carbohydrate addition other than 3 h after feeding with  $\alpha$ -protein, when it was probably due to the larger amount of protein remaining in the stomach.

The insoluble material in the small intestine was little affected either by time or by

the presence of sucrose. The soluble nitrogen content was slightly reduced by the presence of sucrose, possibly because of the preferential uptake of the carbohydrate or the provision of extra energy for amino acid absorption.

Other properties of the proteins when given alone, for instance the slower passage from the stomach of casein and the higher soluble nitrogen concentrations in the small intestine after feeding with  $\alpha$ -protein, were unaffected by the presence of sucrose.

Table 1. *Mean contents with standard errors of soluble and insoluble nitrogen and dry matter (DM) in the stomachs and small intestines of groups of six rats 1, 2 and 3 h after a meal of casein or  $\alpha$ -protein alone and with sucrose, expressed as mg/100 g body-weight*

Diet	Time after meal (h)	Insoluble N	Stomach	
			Soluble N	Insoluble DM
Casein	1	9.19 $\pm$ 0.73	1.98 $\pm$ 0.12	63.5 $\pm$ 5.2
Casein + sucrose	1	14.6 $\pm$ 0.5	2.42 $\pm$ 0.14	102.7 $\pm$ 3.5
Casein	2	6.46 $\pm$ 0.64	1.99 $\pm$ 0.18	46.1 $\pm$ 4.4
Casein + sucrose	2	10.4 $\pm$ 0.6	2.09 $\pm$ 0.16	74.1 $\pm$ 3.8
Casein	3	1.29 $\pm$ 0.35	1.72 $\pm$ 0.23	11.5 $\pm$ 2.66
Casein + sucrose	3	6.76 $\pm$ 0.73	1.90 $\pm$ 0.15	44.5 $\pm$ 3.5
$\alpha$ -Protein	1	7.69 $\pm$ 0.56	2.21 $\pm$ 0.27	60.1 $\pm$ 6.0
$\alpha$ -Protein + sucrose	1	15.5 $\pm$ 0.7	2.57 $\pm$ 0.08	107.8 $\pm$ 4.6
$\alpha$ -Protein	2	3.21 $\pm$ 0.31	1.84 $\pm$ 0.27	23.9 $\pm$ 2.0
$\alpha$ -Protein + sucrose	2	9.49 $\pm$ 0.75	2.35 $\pm$ 0.13	66.1 $\pm$ 4.9
$\alpha$ -Protein	3	0.27 $\pm$ 0.03	0.67 $\pm$ 0.31	3.63 $\pm$ 0.77
$\alpha$ -Protein + sucrose	3	5.49 $\pm$ 0.78	1.87 $\pm$ 0.18	39.1 $\pm$ 5.4
			Small intestine	
Casein	1	0.16 $\pm$ 0.01	2.82 $\pm$ 0.27	3.75 $\pm$ 0.48
Casein + sucrose	1	0.16 $\pm$ 0.01	2.26 $\pm$ 0.15	3.45 $\pm$ 0.30
Casein	2	0.13 $\pm$ 0.02	2.48 $\pm$ 0.34	2.88 $\pm$ 0.21
Casein + sucrose	2	0.19 $\pm$ 0.03	1.87 $\pm$ 0.14	4.00 $\pm$ 0.61
Casein	3	0.12 $\pm$ 0.01	1.96 $\pm$ 0.01	2.47 $\pm$ 0.43
Casein + sucrose	3	0.16 $\pm$ 0.01	1.82 $\pm$ 0.09	3.06 $\pm$ 0.35
$\alpha$ -Protein	1	0.22 $\pm$ 0.03	4.96 $\pm$ 0.32	4.69 $\pm$ 1.18
$\alpha$ -Protein + sucrose	1	0.19 $\pm$ 0.02	3.46 $\pm$ 0.12	3.95 $\pm$ 0.45
$\alpha$ -Protein	2	0.20 $\pm$ 0.02	6.85 $\pm$ 0.37	3.39 $\pm$ 0.31
$\alpha$ -Protein + sucrose	2	0.17 $\pm$ 0.01	4.94 $\pm$ 0.25	3.38 $\pm$ 0.20
$\alpha$ -Protein	3	0.23 $\pm$ 0.11	5.78 $\pm$ 0.41	4.64 $\pm$ 0.75
$\alpha$ -Protein + sucrose	3	0.16 $\pm$ 0.03	4.85 $\pm$ 0.44	2.54 $\pm$ 0.43

#### *Effect of ingestion of different carbohydrates with proteins*

Casein was offered with several different carbohydrates, and cod meal and  $\alpha$ -protein were offered with sucrose and lactose. The nitrogen and dry-matter contents of the stomach and small intestine 2 h after these meals were eaten are given in Tables 2 and 3. The quantity of nitrogen in the stomach varied with the carbohydrate fed in the meal, the differences being almost entirely due to the insoluble fraction. The soluble nitrogen concentrations in stomach and small intestine depended on the proteins in the meal and were little affected by the carbohydrate. The order of effectiveness of the carbohydrates in delaying stomach emptying was (in ascending order) maize starch, maize dextrin, glucose, soluble starch, sucrose and lactose. As Peraino, Rogers, Yoshida, Chen & Harper (1959) observed, the carbohydrates tended to leave the stomach more rapidly than the proteins, although with maize starch this difference was

not marked. Rosenthal & Nasset (1958) also noted variations in stomach emptying with different carbohydrates.

There was a considerable variation in the soluble dry-matter content of both stomach and small intestine with the nature of the added carbohydrate. The insoluble nitrogen concentrations were low and differed little with diet; these proteins had previously been shown to produce little accumulation of insoluble nitrogenous material in the intestine. The rather higher amounts of soluble nitrogen and insoluble nitrogen and dry matter found after a meal of casein with soluble starch probably resulted from the low digestibility of this carbohydrate in the rat.

Table 2. *Mean contents with standard errors of soluble and insoluble nitrogen and dry matter (DM) in the stomachs and small intestines of groups of six rats 2 h after a meal of casein with various carbohydrates, expressed as mg/100 g body-weight*

Carbohydrate	Insoluble N	Soluble N	Insoluble DM	Soluble DM
Stomach				
Maize starch	5.51 ± 1.08	2.02 ± 0.22	62.2 ± 11.2	27.9 ± 4.2
Maize dextrin	7.31 ± 0.32	2.20 ± 0.22	56.7 ± 2.5	44.9 ± 3.9
Glucose	8.10 ± 0.79	1.91 ± 0.12	60.0 ± 5.7	47.8 ± 4.4
Soluble starch	7.57 ± 0.60	2.58 ± 0.13	112.1 ± 9.6	24.9 ± 1.0
Sucrose	10.4 ± 0.6	2.09 ± 0.16	74.1 ± 3.8	68.0 ± 3.5
Lactose	11.2 ± 0.6	2.90 ± 0.26	83.8 ± 4.8	67.7 ± 2.7
Small intestine				
Maize starch	0.19 ± 0.02	2.46 ± 0.16	5.47 ± 0.46	37.6 ± 1.0
Maize dextrin	0.12 ± 0.01	2.38 ± 0.15	2.89 ± 0.26	36.0 ± 2.5
Glucose	0.19 ± 0.02	2.24 ± 0.13	3.77 ± 0.60	31.3 ± 2.1
Soluble starch	0.34 ± 0.02	3.16 ± 0.58	7.48 ± 5.9	37.5 ± 2.4
Sucrose	0.19 ± 0.03	1.87 ± 0.14	4.00 ± 0.61	29.9 ± 1.8
Lactose	0.17 ± 0.02	2.46 ± 0.10	3.93 ± 0.65	76.6 ± 4.5

Table 3. *Mean contents with standard errors of soluble and insoluble nitrogen and dry matter (DM) in the stomachs and small intestines of groups of six rats 2 h after a meal of cod meal or  $\alpha$ -protein alone, and with sucrose or lactose, expressed as mg/100 g body-weight*

Diet	Insoluble N	Soluble N	Insoluble DM	Soluble DM
Stomach				
Cod meal	6.86 ± 0.54	2.01 ± 0.16	49.1 ± 4.6	15.2 ± 1.4
Cod meal + sucrose	6.49 ± 0.53	2.20 ± 0.20	48.2 ± 4.2	61.3 ± 6.1
Cod meal + lactose	8.87 ± 0.61	2.75 ± 0.20	65.2 ± 4.3	66.4 ± 6.5
$\alpha$ -Protein	3.21 ± 0.31	1.84 ± 0.27	23.9 ± 2.0	12.9 ± 1.6
$\alpha$ -Protein + sucrose	9.49 ± 0.75	2.35 ± 0.13	66.1 ± 4.9	64.7 ± 3.7
$\alpha$ -Protein + lactose	10.8 ± 0.9	2.40 ± 0.42	76.1 ± 6.1	73.1 ± 5.5
Small intestine				
Cod meal	0.23 ± 0.03	2.13 ± 0.22	3.96 ± 0.50	28.5 ± 1.6
Cod meal + sucrose	0.18 ± 0.01	2.01 ± 0.23	3.08 ± 0.19	27.5 ± 3.8
Cod meal + lactose	0.16 ± 0.04	1.96 ± 0.18	3.32 ± 0.55	60.4 ± 7.0
$\alpha$ -Protein	0.20 ± 0.02	6.85 ± 0.37	3.39 ± 0.31	58.1 ± 1.9
$\alpha$ -Protein + sucrose	0.17 ± 0.01	4.94 ± 0.25	3.38 ± 0.20	51.9 ± 1.6
$\alpha$ -Protein + lactose	0.18 ± 0.02	4.30 ± 0.18	3.72 ± 0.70	74.2 ± 2.7

*Effect of ingestion of carbohydrates alone*

In order to determine whether the effects of carbohydrates when given with protein could be explained from their behaviour when given alone, three different carbohydrates, maize starch, sucrose and lactose, were given to rats, and the gastro-intestinal nitrogen and dry-matter concentrations are given in Table 4. After feeding with sucrose or lactose, the soluble and insoluble nitrogen concentrations were slightly increased over the fasting values. Although the differences in the dry-matter content of the stomach were not pronounced, maize starch was the slowest to leave the stomach.

The soluble nitrogen contents of the small intestine were increased but little over the fasting levels and seemed to be independent of the carbohydrate. Maize starch produced the highest accumulation of insoluble material.

Table 4. *Mean contents with standard errors of soluble and insoluble nitrogen and dry matter (DM) of the stomachs and small intestines of groups of six rats after fasting for 18 h and 2 h after a meal of carbohydrate alone, expressed as mg/100 g body-weight*

Diet	Insoluble N	Soluble N	Insoluble DM	Soluble DM
	Stomach			
(Fasted)	0.12 ± 0.03	0.15 ± 0.01	2.02 ± 0.42	3.49 ± 0.41
Maize starch	0.11 ± 0.01	0.16 ± 0.01	8.40 ± 1.95	1.89 ± 0.57
Sucrose	0.41 ± 0.08	0.30 ± 0.05	5.65 ± 1.18	4.99 ± 0.77
Lactose	0.35 ± 0.13	0.27 ± 0.02	5.78 ± 1.79	16.9 ± 4.6
	Small intestine			
(Fasted)	0.15 ± 0.01	1.19 ± 0.04	3.89 ± 0.56	27.5 ± 1.1
Maize starch	0.26 ± 0.04	1.34 ± 0.08	9.51 ± 0.82	26.9 ± 1.7
Sucrose	0.28 ± 0.04	1.47 ± 0.14	5.82 ± 0.65	38.2 ± 4.7
Lactose	0.18 ± 0.06	1.47 ± 0.79	3.70 ± 1.02	55.6 ± 17.7

Certain consistencies became apparent: maize starch, sucrose, lactose was the observed order of decrease in insoluble dry matter in the small intestine and increase in soluble dry matter in both stomach and small intestine. In the small intestine the soluble dry matter after feeding with maize starch and the insoluble dry matter after feeding with lactose were close to the respective fasting levels.

In Fig. 1 are shown the Sephadex-ninhydrin profiles of the soluble small-intestine contents of rats after fasting for 18 h and 2 h after eating the carbohydrate meals. The profiles from the carbohydrate-fed animals are clearly similar in nature and level to those from fasted rats, but feeding with sucrose produced slightly more, and feeding with lactose noticeably more, peptides of low molecular weight than did feeding with maize starch.

The ninhydrin profiles for fasted rats and those fed on non-proteinous material resemble those after ingestion of a high-quality well-digested protein (see Zebrowska, 1968) containing little peptide. These profiles represent the digestion of endogenous material, which must be well-digested to prevent excessive nitrogen loss (cf. Dreisbach & Nasset, 1954). It is likely that the increase of peptide material after feeding with sucrose, and especially with lactose, results from the addition to the enzymes of quantities of soluble material with consequent delay in autodigestion.

The delaying effects on stomach emptying of carbohydrates ingested with proteins described above are in the same order as the soluble dry-matter levels in stomach and small intestine when the protein and carbohydrate are given alone. Thus these observations may be explained on the basis that a carbohydrate which is very soluble is preferentially solubilized in the stomach and passes more rapidly into the small intestine, whereas the solubilization of the protein is suppressed and its passage from the stomach delayed.

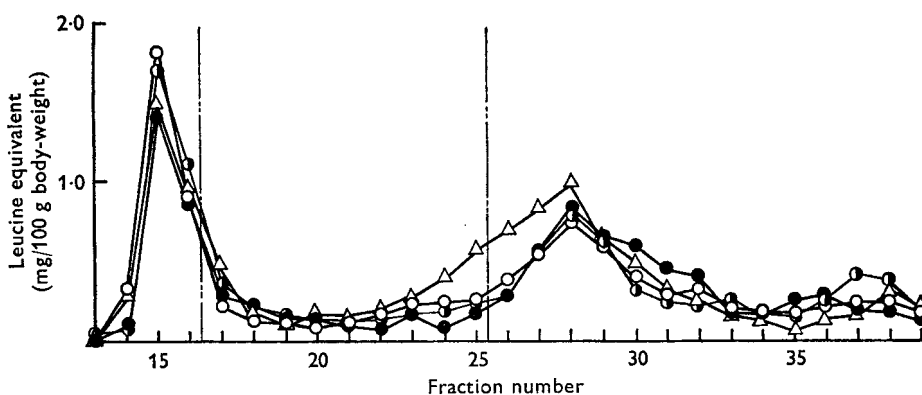


Fig. 1. Fractionation in Sephadex G 25 of the soluble nitrogen of the contents of the small intestine of groups of six rats after 18 h fasting (●—●) and 2 h after a meal of maize starch (●—●), sucrose (○—○) or lactose (△—△).  $\alpha$ -Amino N was measured by reaction with ninhydrin after hydrolysis of the fractions with acid. Values are expressed as leucine equivalents/100 g body-weight. The vertical lines on the abscissa approximately divide protein, peptide and free amino acids.

#### *Plasma amino acid concentrations*

It is well established that feeding with proteins leads to rises in the concentrations of blood plasma amino acid above those found in fasted animals and that these rises are greater in the portal than in the systemic blood (cf. Dent & Schilling, 1949; Dawson & Holdsworth, 1962; Dawson & Porter, 1962).

When casein was given with carbohydrates the amino acid pattern remained much the same but the magnitude of the rise was reduced, particularly in the portal blood (see Table 5). Similar effects were noted by Guggenheim *et al.* (1960). These may be attributed either to the delay in stomach emptying or to competition of the products of carbohydrate digestion for absorption.

#### *General discussion*

As has been shown by other workers (cf. Rogers & Harper, 1966), the presence in the diet of carbohydrate affects the pattern of stomach emptying. From our experiments it appeared that when different proteins were ingested with carbohydrate the behaviour of the meals was largely determined by the properties of the protein, unless the meal was sufficiently large to strain the capacity of the stomach, when the meal was evacuated from the stomach according to mass or, perhaps, volume. The influence of different carbohydrates on the digestion of proteins could be explained by reference to

their solubility within the stomach. It seems probable that some carbohydrates, particularly those which are very soluble but poorly absorbed, may reduce or delay the absorption of a meal by affecting stomach emptying, the absorption of amino acids, and perhaps by affecting the digestive processes by dilution of the enzymes. These factors may also influence the variation in the response of the test animal to the same protein eaten with different carbohydrates, which is discussed by (e.g.) Harper & Katayama (1953) and Register & Peterson (1958), although in their experiments the conditions of feeding were not the same.

Table 5. *Plasma amino acid concentrations in the portal and systemic blood pools of groups of six rats 2 h after a meal of casein alone and with carbohydrates, expressed as  $\mu\text{moles}/100\text{ ml plasma}$*

Amino acid	Portal			Systemic		
	Casein alone	Casein + sucrose	Casein + lactose	Casein alone	Casein + sucrose	Casein + lactose
Asp	7.7	5.3	4.0	3.8	1.1	1.8
Thr	63.0	52.0	39.0	24.2	27.0	24.0
Ser	67.4	104.0	91.0	29.1	49.0	42.0
Glu	64.6	38.0	23.0	26.8	24.0	54.0
Pro	107.8	95.0	71.0	36.3	31.0	25.0
Gly	63.5	55.1	48.0	31.7	26.0	27.0
Ala	221.5	172.0	151.0	62.6	72.0	66.0
Val	70.6	48.6	34.7	488.0	28.5	22.7
Met	15.2	10.9	8.2	7.0	6.1	4.9
Ileu	41.7	29.9	19.7	23.8	13.0	10.0
Leu	58.3	39.3	25.8	37.9	20.2	16.0
Tyr	23.8	19.8	11.7	17.6	12.6	10.1
Phe	20.8	16.9	11.5	11.0	8.2	8.6
Lys	90.8	67.0	51.0	54.6	41.5	44.5
His	24.0	22.1	13.4	10.1	10.0	pr
Arg	21.6	21.0	14.7	17.6	12.6	15.4
Try	13.9	13.3	11.1	10.1	9.3	pr

pr, present.

We are grateful to Mr A. P. Williams and Mr J. E. Cockburn for carrying out amino acid analyses and to Mrs A. Farmer and Miss M. J. A. Medcalf for technical assistance. Thanks are also due to Miss M. Tyler and her staff for care of the rats and to Dr J. E. Ford for helpful discussions.

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