The Assessment of Glucose-Containing Substances in Rumen Micro-Organisms during a Digestion Cycle in Sheep

By P. J. HEALD

Rowett Research Institute, Bucksburn, Aberdeenshire

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It has been established that the digestion of carbohydrates in the ruminant occurs mainly through the agency of the micro-organisms of the alimentary tract. The evidence for this subject has been reviewed by Elsden & Phillipson (1948) and Baker & Harriss (1947–8). Most of this digestion occurs in the rumen. Of the cellulose of the diet about 80 % of the total digested disappears in the rumen, the remainder being digested largely in the caecum (Hale, Duncan & Huffman, 1940, 1947; Gray, 1947).

The nature of the products formed from carbohydrates by microbial attack has been the subject of considerable study and it has been shown by Phillipson (1947–8) and Elsden & Phillipson (1948) that the major portion of these products consists of volatile fatty acids which are absorbed through the rumen wall. It has, however, been suggested by Baker (1942*a*, *b*, 1946) that the substances synthesized by the micro-organisms, such as microbial carbohydrate and protein, as well as the end-products such as the acids formed by fermentation, are also of considerable importance to the host animal.

It appears to be established (see Hungate, 1950) that the breakdown of cellulose by micro-organisms probably involves the following path:

cellulose → cellobiose → (glucose?).

It seems reasonable to suppose that both fermentation and assimilation of cellobiose and glucose can occur simultaneously and, as Elsden (1945) has stated, the problem consists of determining the relative importance of these two processes. Until now no quantitative experiments have been reported concerning the carbohydrate content of the rumen micro-organisms during a digestion cycle in the sheep. This digestion cycle has been described by Tosic (1950).

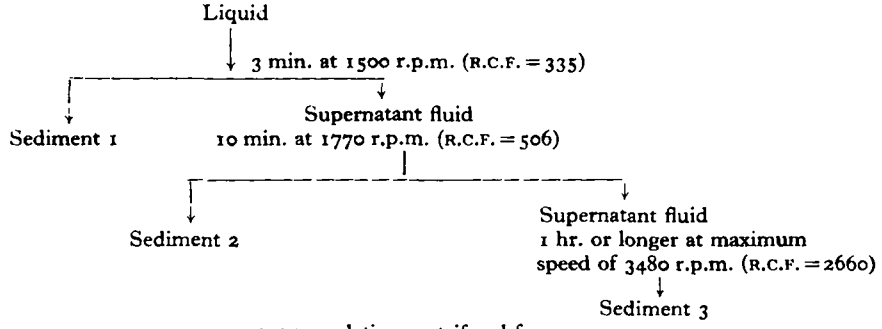
It was shown by Baker & Harriss (1947–8) that not all the micro-organisms of the rumen stain to the same intensity with iodine, and the degree of staining was taken to be a rough indication of polysaccharide synthesis. Since it might be expected that some groups of micro-organisms would have a higher carbohydrate storage than others, the carbohydrate storage of three fractions of rumen micro-organisms from sheep fed on hay was investigated during a 12 hr. digestion cycle. In addition, the quantity of microbial carbohydrate passing out of the abomasum was estimated. This was made possible by using a permanent cannula inserted immediately caudal to the pylorus (Phillipson, 1948).

METHODS

Rumen samples and their fractionation

The rumen samples were collected through an ebonite cannula (Quin, Van der Wath & Myburgh, 1938; Phillipson & Innes, 1939) by means of a wide-bore glass tube. The rumen was massaged before sampling, in order to obtain a more representative sample of the contents (Tosic, 1950).

The samples were collected at intervals of 2, 5, 8 and 12 hr. after a meal of hay, and were strained through S–14 bolting silk (Henry Simon Ltd., Cheadle Heath, Stockport, Cheshire). The strained sample was centrifuged according to the following scheme (Tosic, 1950):



R.C.F.: relative centrifugal force.

In most of the experiments reported below, sediments 2 and 3 were obtained as one fraction.

The sediments were washed with distilled water, and known volumes were hydrolysed with N-HCl for 3 hr. at 100° in sealed tubes. The weight of sediment hydrolysed was obtained by drying known volumes to constant weight at 105°.

Determination of microbial carbohydrate in the rumen and of that leaving the abomasum

Sheep. Two Suffolk Cross ewes were fitted with rumen cannulas and with cannulas inserted into the duodenum immediately caudal to the pylorus, since it is at this point that the most representative sample of digesta leaving the abomasum is obtained (Phillipson, personal communication). A third animal was fitted only with the abomasal cannula.* For comparison of the samples obtained through these cannulas, four Cheviot wethers of the same age received the same diet and were slaughtered at 2, 5, 8 and 12 hr. after feeding. The sheep received feeds of 750 g. chopped hay at intervals of 12 hr. They usually consumed most of the food within 2 hr., and in the first experiments no attempt was made to confine the food and water intake to a 2 hr. period. In later experiments the sheep were trained to consume within 2 hr. the food and as much water as they required. This avoided any change in the rumen micro-organisms during the next 10 hr. due to a fresh influx of food or water. Weights of food and water consumed were recorded.

* The author is indebted to Dr A. T. Phillipson for placing this animal at his disposal.

Samples. The rumen samples were fractionated as already described. Samples from the duodenal cannulas were strained through bolting silk and as much solid material as possible was removed by centrifugation. The sediment was washed with distilled water and again centrifuged at 6000 r.p.m. The resulting sticky sediment was resuspended in water, and known volumes were hydrolysed by the procedure already described. The dry weights of the sediments were obtained by drying known volumes of the suspensions at 105° to constant weight.

In slaughtered animals the rumen and reticulum, the omasum and abomasum were tied off and removed. The contents of the rumen and reticulum were pooled, and samples of the mixed contents were fractionated in the same way as the samples obtained from live animals through a cannula. The contents of the abomasum were removed and sampled, and the samples treated in the same way as those from the duodenal cannula. Total dry weights were obtained on all samples taken. This permitted a check of the constancy of the samples obtained through the cannulas.

Quantitative estimation of carbohydrates. Glucose and 'fermentable reducing substances' in the hydrolysates were estimated by the method described by Heald (1951).

Estimation of reducing substances fermentable by yeast in abomasal fluid. The samples were collected over periods of 12 hr. after feeding, and were strained through bolting silk. The liquid was centrifuged for 1 hr. at 6000 r.p.m. in an angle centrifuge when, in most instances, a clear supernatant liquid was obtained. This was adjusted with sodium hydroxide to pH 6·0–7·0 (Universal Indicator, British Drug Houses Ltd.), and the reducing substances fermentable by yeast were estimated. A preliminary experiment showed that deproteinization of the liquid (Fujita & Iwatake, 1931) was not necessary (Table 1).

Analysis of hay. Dry matter was determined by drying to constant weight at 105°, the ash content by igniting at 500°, cellulose by the method of Crampton & Maynard (1938), nitrogen by the method of Chibnall, Rees & Williams (1943), water-soluble material by extraction with water at 30°. In this last process the residue was washed on a filter until the washings were clear and the total extract evaporated to dryness before it was weighed. Reducing sugars fermentable by yeast and non-fermentable reducing substances were determined in an ethanolic extract of the hay, prepared according to de Mann & de Heus (1949).

Preparation of α-cellulose. α-Cellulose was prepared from a sample of birch-wood pulp, according to the method described by Dorée (1947).

Hydrolysis of cellulose. A sample of birch-wood pulp* of a high α-cellulose content and a sample of an α-cellulose preparation (Dorée, 1947) were washed with warm distilled water and dried. After hydrolysis, the total reducing substances formed were estimated. From the results presented in Table 2 it was concluded that a maximum of 7 % of the cellulose present would be liberated as glucose, assuming that the reducing substance estimated was glucose.

Hydrolysis of hay. A sample of hay was extracted three times with distilled water at 30° for periods of 1 hr. and washed thoroughly on a filter. It was dried overnight at

* Obtained through the courtesy of Dr Rance and Mr Wilson of Messrs Alex. Pirie Ltd., Stoneywood Mills, Bucksburn, Aberdeenshire.

Table 1. *Effect of removal of protein from abomasal liquids of a sheep before estimation of glucose*

(Values expressed in mg./100 ml.)

	Protein removed			Protein not removed		
	Amount of glucose			Amount of glucose		
Solution	Before fermentation (mg.)	After fermentation (mg.)	Difference	Before fermentation (mg.)	After fermentation (mg.)	Difference
Supernatant liquid	19·2	19·3	+0·1	41·8	38·7	−3·1
Supernatant liquid with glucose	21·8	19·3	−2·5	44·4	42·3	−2·1
Glucose	2·6	0·2	−2·4	2·6	0	−2·6

Table 2. *Amount of reducing substances (calculated as glucose) formed on hydrolysis of cellulose under conditions similar to those used for hydrolysis of the sediments from rumen micro-organisms*

		Amount of glucose formed from			
		Birch pulp		α-Cellulose	
Wt. of sample (mg.)	Treatment	(mg.)	(%)	(mg.)	(%)
100·0	Hydrolysed with N-HCl for 3 hr. at 100°	4·5	4·5	—	—
121·9		5·3	4·4	—	—
123·2		5·1	4·2	—	—
303·8		—	—	19·5	6·4
249·7		—	—	17·8	7·1
239·1		—	—	15·8	6·6
280·2	Heated with water for 3 hr. at 100°	—	—	0	0
121·0		0·1	0·1	—	—

105°, and weighed quantities were hydrolysed with N-HCl. Since it was expected that pentoses would be present in the hydrolysate, the reducing substances fermentable by yeast were estimated. The results are given in Table 3.

Table 3. *Amount of fermentable, non-fermentable, and total reducing substances in a hay hydrolysate*

		Reducing substances		
Hay (mg.)	Treatment	Fermentable (%)	Non-fermentable (%)	Total (%)
239·1	Hydrolysed with N-HCl for 3 hr. at 100°	4·2	42·0	46·2
280·2		5·0	45·4	50·4
168·2	Heated with water for 3 hr. at 100°	0	3·9	3·9

In order to obtain some information concerning the nature of the non-fermentable reducing substances, samples of the hydrolysates were neutralized with silver carbonate. The clear supernatant fluids obtained on centrifuging were spotted on to Whatman no. 1 paper and the chromatograms were developed with a mixture of *n*-butanol, acetic acid and water, and also with phenol (Partridge, 1948) and with a mixture of pyridine-*n*-amyl alcohol and water (Werner & Odin, 1949) using glucose, arabinose and xylose as reference sugars.

A faint glucose spot and an intense xylose spot were detected when the dried chromatograms were sprayed with ammoniacal silver nitrate (Partridge, 1948).

Qualitative analysis of the water-soluble sugars in the hay. Hay (20 g.) was extracted four times at 37° for periods of 30 min. with 100 ml. water. The combined extracts were evaporated to a small volume (20 ml.) at 40° under reduced pressure, and 4 vol. 95 % (v/v) ethanol were added. The precipitate obtained was centrifuged and the supernatant liquid was removed and evaporated to dryness at 40° under reduced pressure. The dry residue was dissolved in 0·5 ml. distilled water and the solution was spotted on to Whatman no. 1 paper together with a solution of glucose, sucrose and fructose as reference sugars. The chromatograms were developed for 72 hr. in *n*-butanol water, dried at 105° and sprayed with naphthoresorcinol (Forsyth, 1948) to detect fructose and sucrose, and with aniline hydrogen phthalate (Partridge, 1949) to detect glucose. An intense fructose spot and a faint glucose spot were observed.

The precipitated material (50 mg.) was hydrolysed in a sealed tube with 0·1 N-H_2SO_4 (1 ml.) for 1 hr. at 100°. Not all the precipitate dissolved. The hydrolysate was adjusted to pH 4·5 (Congo red) by addition of barium carbonate and centrifuged. The clear supernatant fluids were spotted on to paper and the chromatograms developed with *n*-butanol water for 72 hr. Glucose and fructose were included as reference sugars. The chromatograms were dried at 105° and sprayed with naphthoresorcinol and aniline hydrogen phthalate. Fructose was the main carbohydrate detected, but there were also some red streaks leading downwards from the point of application of the hydrolysate. These might have been fructose polymers incompletely hydrolysed.

RESULTS

Glucose contents of sediment 1 and of sediments 2 and 3 together. The results of two determinations of glucose and of fermentable and non-fermentable reducing substances on sediment 1 and sediments 2 and 3 are shown in Fig. 1. Essentially similar graphs were obtained by analysis of sediments from two other sheep. The contents of glucose and of fermentable reducing substances were in close agreement at all stages of the digestion cycle. The content of non-fermentable reducing substances varied a little throughout the cycle and was the same for both sediments.

The content of glucose in hydrolysates from sediment 1 was consistently higher than that of glucose in the hydrolysates from sediments 2 and 3. It was thought that this difference might be due to glucose produced by the hydrolysis of cellulose or similar materials contained in the sediment as plant particles, since it is known that plant particles were present (Tosic, 1950). Experiments were therefore carried out to determine the cellulose content of the sediment and the results (Table 4) were compared with those of hydrolysis of cellulose (Table 2) and of hay (Table 3) already reported.

Cellulose in sediment 1. Cellulose was estimated in an acetone powder (see Heald, 1951) of sediment 1, but no correction was made for the ash content. From the results in Tables 2 and 4, it was calculated that the maximum quantity of glucose that might be produced from hydrolysis of the cellulose residues in sediment 1 was 0·5–0·6 %. The glucose values shown for sediment 1 in Fig. 1 would, therefore, appear to be too high by this amount.

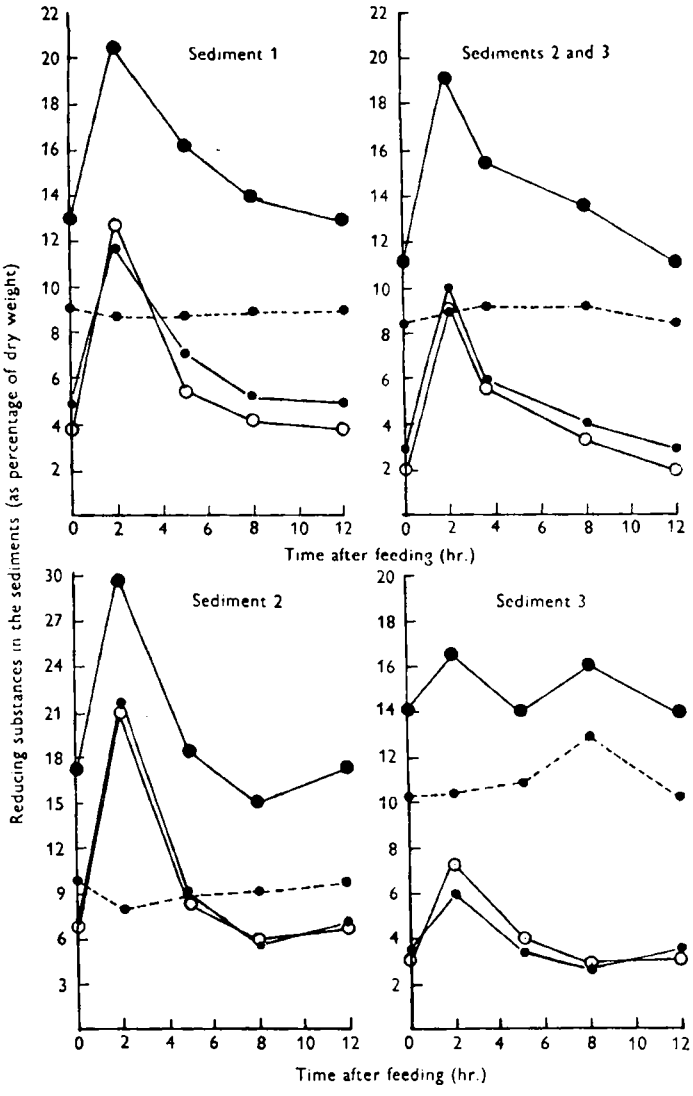


Fig. 1. Changes in the quantities of reducing substances in the sediments centrifuged from rumen liquor. Results are shown for sediments 1, and 2 and 3 together, for one sheep and for sediments 2 and 3 separately, for another sheep. ●——●, total reducing substances; ○——○, glucose-containing carbohydrate; •——•, fermentable reducing substances; •– – –•, non-fermentable reducing substances.

Table 4. *Cellulose content of acetone powder of sediment 1 from rumen micro-organisms*

Exp. no.	Sediment taken (mg.)	Cellulose residue* mg.	Cellulose residue* As percentage of sediment
1	225·5	17·9	7·9
2	273·0	24·5	8·9
3	339·6	29·3	8·6

* Not corrected for ash content.

Glucose contents of sediments 1, 2, 3 *and the cellulose content of sediment* 1 *determined concurrently*. To correlate the previous results the contents of glucose were estimated in the three sediments and the content of cellulose in sediment 1 from one sheep. The feed for the sheep consisted of 1180 g. chopped hay given in two portions of 590 g. at 12 hr. intervals. The hay contained (on a dry-weight basis) 14·3 % water-soluble matter, 0·45 % total reducing substances calculated as glucose, and 0·33 % yeast-fermentable reducing substances calculated as glucose (Tosic, personal communication). The animal consumed all the food within 2 hr. The results for sediment 1 in this experiment were essentially the same as those obtained for sediment 1 in a previous experiment and recorded in Fig. 1. The results for sediments 2 and 3 in the present experiment are also shown in Fig. 1.

From the dry weights of the sediments obtained from the rumen samples it was calculated that the dry weights of the rumen fractions in mg./100 g. wet rumen sample were of the following order: sediment 1, 500–600; sediment 2, 60–100; sediment 3, 250–300.

It was further assumed that the rate of passage of material out of the rumen into the abomasum was of the same order as that of material passing out of the abomasum into the duodenum, i.e. 400 ml./hr. This figure is probably high (Phillipson, Green, Reid & Vowles, 1949), but calculated in this way, the quantity of glucose-containing substances in the micro-organisms passing out of the rumen in 24 hr. was of the order of 5 g.

Glucose liberation in the abomasum. Consideration of the above calculation suggested at least three criticisms that could be applied to it and might account for the low value for glucose-containing substances. First, no assessment could be made of the glucose-containing substances in the micro-organisms adhering to the plant particles (Baker & Harriss, 1947–8), removed on straining the rumen liquor. Secondly, the relatively long centrifuging procedure results in a rise in temperature and could have led to autolysis of the fractions. Thirdly, since the samples were obtained through a cannula, calculations based on dry-matter content of the liquid sample cannot be directly applied to the actual rumen contents.

In order to overcome the second and third objections, an attempt was made to estimate the glucose-containing substances in the sediment obtained from the abomasum, and in the partly digested food leaving the abomasum. To get a comparison with the results obtained with samples removed through the cannulas, sheep were slaughtered at intervals after feeding and samples of the rumen and abomasal contents were prepared as described on p. 86.

It has been suggested (cf. Baker, 1946) that in the abomasum the micro-organisms entering from the rumen are broken down. Since this may also involve a breakdown of microbial carbohydrate, an estimation of the carbohydrate alone in any sediment of micro-organisms obtained would give a wrong value for the quantity of such carbohydrate passing through to the rest of the alimentary tract. The results of estimating reducing substances fermentable by yeast in the liquid leaving the abomasum are presented in Table 5, and show that scarcely any fermentable reducing substances were liberated in the abomasum. The carbohydrate content of micro-organisms leaving the abomasum and of those in the rumen were then estimated as already described.

Table 5. *Fermentable reducing substances in abomasal liquid of a sheep*

(Values expressed in mg./100 ml.)

Exp. no.	Amount formed: 12 hr. after feeding	2 hr. after feeding	5 hr. after feeding	8 hr. after feeding
1	1·5	1·6	1·2	2·2
2	2·5	4·1	1·8	6·5

Glucose content of the microbial sediment leaving the abomasum. The results obtained from the two cannulated animals and from the slaughtered animals are presented in Fig. 2. Only the content of glucose is shown, though in all instances those of yeast-fermentable and non-fermentable reducing substances were estimated and were found to correspond to the values shown in Fig. 1.

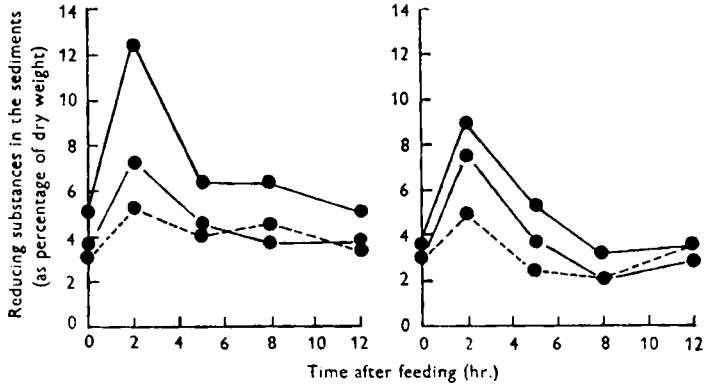


Fig. 2. Changes in the quantities of reducing substances in the sediment centrifuged from rumen and abomasal liquor. Results are shown for samples taken through rumen and duodenal cannulas, and for samples taken from slaughtered sheep. ●——●, carbohydrate in sediment 1; ● — ●, carbohydrate in sediments (2 and 3); ●- - -●, carbohydrate in the sediment from the abomasum.

The average dry weight of the total sediment centrifuged from the strained abomasal samples was 0·8–1·0 % of the wet sample. By using this figure, by taking the average amount of glucose-containing material in this sediment as 5 %, and by assuming that the rate of passage of food through the abomasum is 400 ml./hr. (Phillipson *et al.* 1949), it was calculated that the quantity of glucose in combined form in the micro-organisms passing into the duodenum was probably of the order of 5 g./24 hr.

DISCUSSION

This paper deals with two aspects of carbohydrate synthesis and storage by the rumen micro-organisms. The most definite fact arising is that for sheep fed on hay the quantity of glucose-containing carbohydrate stored in the micro-organisms and passing into the duodenum is nutritionally insignificant. On a hay diet, the main nutritional value of the carbohydrate broken down by the micro-organisms of the rumen lies in its subsequent fermentation to fatty acids (cf. Marston, 1948). That this fermentation may be preceded by a storage of carbohydrate within the micro-organisms in the rumen

does not affect this conclusion, since utilization of the stored carbohydrate by the micro-organisms may again result in further production of fatty acids. Work to investigate this problem is in progress.

The second point arising concerns the rapid increase in carbohydrate content of the micro-organisms immediately after a meal, followed by an equally rapid decline. This change is especially pronounced with sediment 2, the percentage of carbohydrate rising from 6·5 to 21–22 in 2 hr. and falling to 9 within 5 hr. after feeding (Fig. 1). A possible explanation of this increase might be that it is due mainly to the influx of soluble carbohydrates in the food, which in the hay used consisted mainly of fructose and fructosans. It is to be noted, however, that on a total quantity basis sediment 1 accounted for the greater amount of carbohydrate stored. These figures suggest that the different groups of micro-organisms of the rumen assimilate the soluble sugars to different degrees, but further experiments are necessary to decide this and to assess the significance, if any, of such storage. The close agreement observed between the yeast-fermentable reducing substances and the glucose content of the sediments suggests that for sheep fed on a hay diet this method, rather than the more prolonged chromatographic procedure, might be used in preliminary experiments.

The almost constant content of non-fermentable reducing substances has been discussed in a previous paper (Heald, 1951). It is of interest that this fraction is present to the same extent in the micro-organisms obtained from the abomasum as in those from the rumen. This would suggest that if such substances are liberated by digestion of the micro-organisms, the process must take place lower down in the alimentary tract.

The amounts of total reducing substances estimated are great and, if taken to indicate glucose, would lead to a wrong assessment of the value of microbial carbohydrate to the animal. For example, the total reducing substances in the abomasal hydrolysates ranged from 13 to 15 % of the dry weight of the micro-organisms. Taking this figure as the glucose content, the calculation applied on p. 91 would lead to a figure of 13–15 g./24 hr., whereas the value is more likely to be 5–6 g. This error is due in part to the use of the Hagedorn-Jensen reagent, but it has been shown (Heald, 1951) that with the Somogyi (1945) reagent the error in estimating the quantity of carbohydrate in hydrolysates of rumen micro-organisms would be greater.

SUMMARY

1. A study has been made of the carbohydrate content of the rumen micro-organisms during a digestion cycle in sheep. Samples of liquid from the rumen and abomasum were obtained from slaughtered animals and also by cannulas from live animals.

2. The average amount of glucose in the micro-organisms from the abomasum of hay-fed sheep was 4–5 % of the dry weight, and the total quantity passing from the abomasum was calculated to be 5–6 g./24 hr. This quantity can be of little importance to the sheep.

3. The storage of glucose-containing carbohydrate within the rumen by three fractions of micro-organisms was studied and was found to undergo a marked increase immediately after a meal. This increase was followed by a rapid fall.

4. It is pointed out that the use of reducing methods for estimation of sugars in hydrolysates of micro-organisms can lead to a false assessment of their carbohydrate content.

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REFERENCES

Baker, F. (1942*a*). *Nature, Lond.*, **149**, 582.
Baker, F. (1942*b*). *Nature, Lond.*, **150**, 479.
Baker, F. (1946). *Nature, Lond.*, **158**, 609.
Baker, F. & Harriss, S. T. (1947–8). *Nutr. Abstr. Rev.* **17**, 3.
Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
Crampton, E. W. & Maynard, L. A. (1938). *J. Nutrit.* **15**, 383.
de Mann, T. & de Heus, J. G. (1949). *Rec. Trav. chim. Pays-Bas*, **68**, 43.
Dorée, C. (1947). *Methods of Cellulose Chemistry*, 2nd ed., p. 363. London: Chapman and Hall Ltd.
Elsden, S. R. (1945). *J. exp. Biol.* **22**, 51.
Elsden, S. R. & Phillipson, A. T. (1948). *Ann. Rev. Biochem.* **17**, 705.
Forsyth, W. G. C. (1948). *Nature, Lond.*, **161**, 239.
Fujita, A. & Iwatake, D. (1931). *Biochem. Z.* **242**, 43.
Gray, F. V. (1947). *J. exp. Biol.* **24**, 15.
Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
Hale, E. B., Duncan, C. W. & Huffman, C. F. (1940). *J. Dairy Sci.* **23**, 953.
Hale, E. B., Duncan, C. W. & Huffman, C. F. (1947). *J. Nutrit.* **34**, 747.
Heald, P. J. (1951). *Brit. J. Nutrit.* **5**, 75.
Hungate, R. E. (1950). *Bact. Rev.* **14**, 1.
Marston, H. R. (1948). *Biochem. J.* **42**, 564.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
Phillipson, A. T. (1947–8). *Nutr. Abstr. Rev.* **17**, 12.
Phillipson, A. T. (1948). *J. Physiol.* **107**, 21*P*.
Phillipson, A. T., Green, R., Reid, R. S. & Vowles, L. E. (1949). *Brit. J. Nutrit.* **3**, iii.
Phillipson, A. T. & Innes, J. R. M. (1939). *Quart. J. exp. Physiol.* **29**, 333.
Quin, J. I., Van der Wath, J. G. & Myburgh, S. (1938). *Onderstepoort J. vet. Sci.* **11**, 341.
Somogyi, M. (1945). *J. biol. Chem.* **160**, 61.
Tosic, J. (1950). Personal communication.
Werner, I. & Odin, L. (1949). *Uppsala LäkFören. Förh.* **54**, 69.