The fermentation of soluble carbohydrates in rumen contents of cows fed diets containing a large proportion of hay

By J. D. SUTTON

National Institute for Research in Dairying, Shinfield, Reading

(*Received* 2 *April* 1968—*Accepted* 21 *June* 1968)

1. Studies were made of the fermentation of D-glucose, D-fructose, D-galactose, D-xylose, L-arabinose and sucrose by rumen contents from two cows given 70% hay and 30% dairy cubes once daily.

2. In in vivo experiments the monosaccharides only were infused into the rumen for 8 h at 200 g/h. Glucose and fructose were almost completely fermented but the other carbohydrates were fermented more slowly. Changes in the proportions of volatile fatty acids (VFA) in the rumen indicated marked differences among the carbohydrates in the proportions of VFA produced.

3. In in vitro experiments all the carbohydrates were incubated for 2 h with 150 g mixed rumen contents. The carbohydrates were added at 10 min intervals at a rate equivalent to that used in vivo. Between 97% and 100% of the sucrose, glucose and fructose were fermented, but only 42–56% of the other carbohydrates. Acetic acid was the predominant acid produced, especially from galactose and the pentoses. Propionic acid constituted less than 20% of the VFA produced from galactose but about 30% from the other carbohydrates. n-Butyric acid formed about 20% of the VFA from the hexoses and sucrose but only 10% from the pentoses. Appreciable amounts of lactic acid were produced from glucose and fructose only.

4. Net recovery of carbon from fermented carbohydrate in VFA plus lactic acid was about 35%; if results were not corrected for VFA produced in control flasks, the resulting gross recovery was 50–63%. The relative validity of the two terms is discussed.

5. There was good qualitative agreement between results from in vivo and in vitro experiments and this was taken as evidence of the general validity of the in vitro technique. Only the development of accurate methods for determining rates of fermentation in vivo would allow evaluation of the technique in absolute terms. Such a comparison would be of great value.

6. The relevance of the results to the fermentation of the carbohydrates of feeds is considered. The differences found between the fermentation products of sucrose and those of glucose and fructose demonstrate that it is not valid to extrapolate results obtained with monosaccharides to their related polysaccharides without experimental evidence.

The carbohydrates of the natural feeds of ruminants, while often present in complex form, are built from relatively few monosaccharides. Bailey (1962) has listed the most common in plants as being, in decreasing order of abundance, glucose, fructose, xylose, galactose, arabinose, galacturonic acid and glucuronic acid. The classic work of Phillipson & McAnally (1942) clearly showed not only that carbohydrates were fermented in the rumen to volatile fatty acids (VFA) but that such similar substrates as mono- and di-saccharides were fermented at very different rates. Later workers confirmed and extended these results, showing differences among monosaccharides in the proportions of VFA produced by the fermentation. Since there is now strong evidence that the efficiency with which VFA support various productive processes in the ruminant varies according to the proportions of VFA that are produced (see Blaxter, 1962), it is important that the fermentation in the rumen be well understood. Unfortunately, despite the large number of experiments that have been conducted, there is still much disagreement about the relative proportions of VFA that are produced from different carbohydrates.

Furthermore, there is a serious lack of information on the quantitative aspects of the fermentation of these carbohydrates. Most in vivo experiments conducted to date have permitted comparisons among carbohydrates but have provided no information about absolute rates of fermentation. In vitro experiments give a greater possibility of determining absolute rates of VFA production but the extent to which the results can be applied to the in vivo situation is limited by the artificial nature of the conditions under which measurements are made. This is especially so where such preparations as washed cell suspensions (Doetsch, Robinson, Brown & Shaw, 1953; Robinson, Doetsch, Sirotnak & Shaw, 1955) or long incubation times (Elsden, 1945) are used. Only Hueter, Gibbons, Shaw & Doetsch (1958) and Elsden (1945) appear to have attempted direct comparison of in vivo and in vitro techniques for studying the conversion of soluble carbohydrates into VFA.

The purpose of the experiments described below was to study the metabolism of five monosaccharides—glucose, fructose, galactose, xylose and arabinose—and one disaccharide—sucrose—under carefully controlled conditions in vivo and in vitro in an attempt to provide a more satisfactory basis for comparison of these carbohydrates than has been provided previously. In particular, it was hoped to provide more precise information about the quantitative aspects of the conversion of carbohydrates into VFA.

EXPERIMENTAL

Animals and management

Two dry Friesian cows, Adelaide and Desmine, each with a permanent rumen fistula closed with a rubber cannula and bung (Balch & Cowie, 1962), were used in all experiments. They were housed in metabolism stalls. In Expt 1 they were offered 5 kg meadow hay and 2 kg dairy concentrate cubes; in later experiments the same feeds were offered at 80% of the level offered in Expt 1 since the cows gained too much weight in that experiment. Feeds were offered once daily at 17.00 h and were consumed within 3 h. Commercial salt licks providing a wide range of minerals (Mineral Salt Licks; Bell and Son Ltd, Liverpool) were available throughout. At all times except during experimental infusions, drinking water was available and, in addition, water was infused into the rumen by gravity flow at 68 l./day to reduce the sudden fluctuations in the volume of rumen contents due to drinking. The rate of infusion was that used in other experiments at this Institute with milking cows (Rook & Balch, 1961).

In vivo experiments

Infusions. Five monosaccharides were studied: D-glucose (Cerelose, dextrose monohydrate; Brown and Polson Ltd, London); D-fructose (levulose, C.P. Special; Pfanstiehl Laboratories Inc., Waukegan, Illinois); D-galactose (anhydrous D(+)galactose; Thomas Kerfoot and Co. Ltd, Vale of Bardsley, Lancs.); D-xylose (Dr Theodor Schuchardt, GMBH and Co., Munich); and L-arabinose (L-Arabinose, N.F., Pfanstiehl Laboratories Inc.). The monosaccharides were dissolved in water and the resultant solution was infused at 2 l./h for 8 h. In Expt 1 the metabolism of all the monosaccharides when infused at 200 g/h was compared; the order of infusion is

shown in Table 2. Water (control) was infused twice and glucose three times to gain some measure of the repeatability of response. The other monosaccharides were infused once only into each cow. The purpose of Expt 2 was to examine the metabolism of one sugar, glucose, when infused at 0, 35, 90, 145 or 200 g/h, once at each level into each cow. The order of infusion is shown in Table 5. A period of at least 8 days was allowed between each infusion to reduce carry-over effects.

The solutions were infused through rubber tubes passing through the bung of the rumen cannula; the site of infusion was about 7 cm below the surface of the rumen contents immediately below the fistula. The rate of infusion was controlled by a D.C.L. Series II Micro-pump (F.A. Hughes and Co. Ltd, Epsom).

Sampling. Samples were withdrawn from the rumen by gentle suction through a wire-gauze filter anchored in the rumen with a 1 kg weight. Two such filters were used, one on the bottom of the ventral sac and the other in the reticulum.

Samples of 60 ml were withdrawn from each site and mixed at once. The pH was determined within 2 min of sampling with a Pye Universal pH meter with a Pye-Ingold combined glass and reference electrode.

In Expt 1 samples were placed in storage at $-17°$ immediately after the pH had been measured. In Expt 2 attempts were made to reduce further the possibility of metabolism occurring after the sample was removed from the rumen. To 10 ml of the freshly taken sample were added $Ba(OH)_2$ and $ZnSO_4$, as described below, in preparation for analysis for soluble carbohydrates; this sample was stored at 3° and analysed within 48 h. To 100 ml of the remaining sample were added 5 ml 5% (w/v) $HgCl_2$; this sample was stored at $-17°$ for all the other analyses.

Samples were analysed for concentrations of VFA, lactic acid and carbohydrate (as total reducing substances—TRS). In Expt 1 proportions of acetic, propionic, iso- and n-butyric and iso- and n-valeric acids were determined. In Expt 2 the valeric acids were not determined, because changes in the proportions of these two acids were small and irregular in Expt 1; a mean value was calculated from the results of Expt 1 and applied to the results for Expt 2. In Expt 1 only, the starch content of the samples was estimated.

In vitro experiments

About 2 kg mixed rumen contents were removed from the centre of the rumen and taken in warmed containers to the laboratory. In preliminary experiments it was found that the rate of fermentation of glucose was greater in whole rumen contents than in the liquid phase alone. To obtain similar amounts of solid and liquid phase in each flask the two phases were first separated by squeezing total contents through two layers of surgical gauze. The contents were then rapidly reconstituted by measuring 120 ml liquid phase and 30 g of the solid retained on the gauze into each of the fourteen 250 ml round-bottomed flasks in which the incubations were conducted. These amounts of the two phases resulted in approximately the same dry-matter content in vitro as was found in vivo. The flasks were stoppered with rubber bungs fitted with a syringe needle and a bunsen valve and were placed in water at 39° in a modified manometer bath. When all the flasks were prepared they were gassed with carbon dioxide for about 5 min. The gas lines were then disconnected and 10 ml disposable

plastic syringes containing substrate were fitted in the needles in the stoppers. Shaking at about 110 strokes/min was then commenced. About 30 min was required between taking the sample from the rumen and beginning the shaking; a further 10 min was allowed before substrate additions began.

The rate of addition of substrates was related to that used in the in vivo experiments. The weight of contents in the rumen was determined by removal of all the contents once from each cow; the contents were weighed, sampled for determination of dry-matter content, and returned to the rumen. To simulate continuous infusion, 2 ml of the substrate solutions were added at 10 min intervals through the 2 h incubation. Substrates were dissolved in artificial saliva (McDougall, 1948). Each substrate was incubated in duplicate flasks. Two flasks (0 h) were removed at the start of the incubation; artificial saliva alone was added to two further flasks (control) throughout the incubation.

At the end of the 2 h incubation, the flasks were removed. About 30 ml of contents were squeezed through one layer of surgical gauze and were prepared for analysis as described above for Expt 2. Samples were analysed for concentrations of VFA, lactic acid and TRS and for proportions of VFA. The pH of the remaining contents of the flasks was measured. The weight and dry-matter percentage of the contents in the flasks were determined and the liquid volume was calculated.

Three experiments were conducted using the in vitro technique. Expt 3 was an attempt to repeat Expt 1 in vitro, the fermentation of the same five monosaccharides being studied. Each monosaccharide was added at 375 mg/h, a level equivalent to the 200 g/h used in vivo. Two incubations were made with digesta from each cow. In Expt 4, the metabolism of sucrose (Analar grade, British Drug Houses Ltd, Poole) was compared with that of glucose; both carbohydrates were added at the same level (on a carbon basis) as was used in Expt 3. In addition the metabolism of glucose, galactose and xylose was studied when they were added at 187·5 mg/h—half the rate used in Expt 3. Incubations were made on only one occasion with each cow. In Expt 5 the effect of an extended period of incubation was examined. Glucose and xylose were added at 187·5 mg/h for 2 h in duplicate; control flasks were also incubated in duplicate. At the end of 2 h, one of each duplicate set of flasks was removed. No further substrate was added after 2 h but at 3 and 3·5 h, 6 ml of artificial saliva were added to the remaining flasks to increase the buffering capacity of the contents. At the end of 4 h the remaining flasks were removed. The incubations were conducted on two occasions with contents from cow Adelaide.

The amounts of VFA produced in vitro are presented in two forms. Gross production is calculated from the increase in the amount of VFA in each flask during the 2 h incubation. Net production is gross production less production in the control flasks to which no carbohydrates were added; it represents an attempt to distinguish between the VFA produced from added carbohydrate and that produced by fermentation of feed in the inoculum. No lactic acid was produced in control flasks, so no such correction was required for that acid.

Samples from Expts 3 and 4 were analysed for molar proportions of acetic, propionic, iso- and n-butyric and iso- and n-valeric acids. Only small and variable changes

were detected in the amounts of iso-butyric acid and iso- and n-valeric acids during the incubations. The maximum gross production of these three acids was only 1·0% of the production of all the VFA. In calculating the amounts of individual VFA produced, it was therefore assumed that the amounts of iso-butyric and iso- and n-valeric acids remained unchanged during the incubation; production of acetic, propionic and n-butyric acids only was calculated.

Statistical analysis. In Expt 3 statistical analysis for each variate was carried out separately for each cow and the standard error of a difference between substrate means was derived from the interaction of substrates and occasions for the particular animal. Similarly, when referring to substrate values averaged over both cows, the error has been derived from a pooled estimate of the interaction of substrates and occasions within cows. Although in this experiment combined analyses of variance for both cows never showed significant interaction between substrates and cows, a sample of only two animals was considered inadequate to establish the general reproducibility of the results with confidence. Because of the type of standard error chosen, conclusions are only strictly appropriate for those particular animals, although much wider validity may well apply.

In Expt 4, since incubations were conducted on only one occasion with each cow, the standard error of substrate differences for a given cow was necessarily based on the mean square between duplicate incubations. Similarly, when discussing substrate values averaged over both animals, the error was derived from a pooled estimate of the mean square between duplicates within cows. These error variances tend to underestimate those available in Expt 3 and conclusions in Expt 4 strictly apply only to the particular animals on their single occasions though again much wider validity may well apply.

In neither Expt 3 nor Expt 4 were the amounts of VFA or lactic acid produced analysed statistically as they were of limited value in view of the wide range in the amount of substrate metabolized. The conversion of metabolized substrate into fermentation products was more meaningful, and this relationship was examined statistically.

Analytical procedures

Rumen liquor was centrifuged for 40 min at 2500 ***g*** and analysed for total VFA by steam distillation (Annison, 1954) and lactic acid by the technique of Elsden & Gibson (1954). Proportions of VFA were determined by gas chromatography. In Expts 1 and 2 the procedure of Tilley, Canaway & Terry (1964) was used. In Expts 3 and 4 samples were analysed on an Aerograph-600-C gas chromatograph with a hydrogen-flame ionization detector (Hodson, McGilliard, Jacobson & Allen, 1965). Initially samples were prepared for analysis on the Aerograph gas chromatograph by the procedure of A. D. McGilliard (personal communication) in which the sample is deproteinized by the addition of 25% (w/v) metaphosphoric acid before injection. When the sample prepared in this way contained high concentrations of certain of the carbohydrates, interfering peaks were detected on the chromatogram. To remove the carbohydrate, all samples in Expts 3 and 4 were extracted into diethyl ether by the procedure of Storry & Millard (1965). They were then re-extracted into water before injection since

the large diethyl ether peak obscured the VFA peaks when the samples were injected in ethereal solution.

The solid fraction resulting from centrifuging the rumen contents was examined for starch in Expt 1. It was suspended in water and the depth of purple colour resulting from the addition of iodine in potassium iodide solution was estimated on a scale of 0 (no colour) to 3 (almost black).

Soluble carbohydrates were determined as total reducing substances (TRS) by the technique of Somogyi (1945, 1952) after precipitating the protein in 1 vol. of rumen contents by the addition of 1 vol. 0·3 N-$Ba(OH)_2$ and 1 vol. 5% (w/v) $ZnSO_4.7H_2O$ solution. Slightly different conditions were required for analysis of different carbohydrates. Sucrose was determined by the same technique after hydrolysis by 0·17 N-H_2SO_4 at 100° for 3 min.

RESULTS

Preliminary experiments

Level of infusion. In preliminary trials glucose was infused at 200, 300 and 400 g/h for 6 h to determine the maximum level that could be infused without accumulation of lactic acid. At the two higher levels of infusion the concentrations of both lactic acid and glucose increased markedly and were still rising at 6 h. At the lowest level of infusion, neither metabolite was found in appreciable quantities; this level was therefore selected for infusion of all carbohydrates in Expt 1. The rate of change of rumen pH and of the concentration and molar proportions of VFA was small by 6 h, when glucose was being infused at 200 g/h; in order to give a rather greater opportunity for a steady-state situation to be attained, all infusions were continued for 8 h in Expt 1.

Site of sampling. Initially glucose was infused into the ventral sac of the rumen and samples were taken from the reticulum and from a point in the ventral sac about 35 cm away from the site of infusion. No consistent difference in pH or concentration of VFA between the two sampling sites was detected but glucose tended to accumulate in the ventral sac occasionally; in one trial the concentration of glucose reached 480 mg/100 ml in the ventral sac whilst less than 1 mg/100 ml was present in the reticulum. In an attempt to distribute the infusate more uniformly throughout the reticulo-rumen, the site of infusion was moved to a position about 7 cm below the surface of the rumen contents and immediately ventral to the fistula. Samples were taken from near the bottom of the ventral sac and from the reticulum. During two infusions of glucose into each cow, samples from the two sampling sites were analysed separately for pH, TRS and concentration and proportions of VFA. Differences between sites were small and irregular. In all subsequent infusions, samples were taken from both sites and mixed in equal volumes before being analysed.

Expt 1. Different carbohydrates in vivo

Basal fermentation. The small variation in the proportions of VFA found in the rumen at the start of each infusion (Table 1) is evidence that the basal fermentation was very similar both from day to day and between cows. Rather more variation was apparent in concentrations of VFA. When water alone was infused, a steady fall in the

concentration of VFA and a rise in pH occurred, reflecting the declining rate of fermentation of the previous feed (Tables 2 and 3). Changes in the proportions of VFA between the start of the infusion and the end, 8 h later, were small (Table 3). Thus a relatively simple fermentation pattern was established for the study of the effect of added carbohydrates.

Disappearance of carbohydrates. As in the preliminary trials, the infusion of glucose at 200 g/h resulted in the accumulation of negligible amounts of TRS in the rumen

Table 1. *Expt 1: mean and range of the pH and the concentrations and molar proportions of volatile fatty acids (VFA) in the rumen of both cows before the start of infusions*

			Molar %					
	pH	VFA (m-moles/l.)	Acetic	Propionic	Iso-butyric	n-Butyric	Iso-valeric	n-Valeric
			Cow Adelaide					
Mean	6·4	75·3	70·5	17·8	0·6	9·6	0·8	0·7
Range	6·2–6·8	67·9–80·7	69·6–71·7	16·8–18·6	0·4–0·8	9·0–10·4	0·5–1·0	0·5–0·9
			Cow Desmine					
Mean	6·8	58·5	70·8	17·1	0·9	9·4	1·1	0·7
Range	6·7–6·9	52·9–63·6	68·2–72·3	16·3–18·7	0·7–1·0	8·4–10·5	0·9–1·4	0·5–0·9

Table 2. *Expt 1: difference between rumen samples taken at the start and after 8 h of infusion in pH and concentrations of total reducing substances (TRS), volatile fatty acids (VFA), lactic acid and starch in both cows.*

(All carbohydrates were infused at 200 g/h for 8 h)

Infusate*	pH	TRS (mg/100 ml)	VFA (m-moles/l.)	Lactic acid (m-moles/l.)	Starch†
		Cow Adelaide			
(3) Water	+0·4	−1·3	−15·6	ND	0
(9) Water	+0·5	−1·1	−18·1	ND	ND
(1) Glucose	−0·2	+8·2	+14·9	ND	ND
(2) Glucose	−0·4	+14·7	+11·8	+0·2	+3
(8) Glucose	−0·2	+10·2	+11·7	0	+3
(5) Fructose	−0·4	+11·3	+9·3	+4·3	+3
(7) Galactose	−0·4	+80·8	+16·1	+0·3	+2
(4) Xylose‡	−0·4	+146·7	+9·5	+0·2	+1
(6) Arabinose	−0·4	+171·7	+9·3	+0·2	+1
		Cow Desmine			
(3) Water	+0·3	+1·6	−12·7	ND	0
(9) Water	+0·3	−1·9	−10·2	ND	ND
(1) Glucose	−0·3	+2·3	+19·0	ND	ND
(2) Glucose	−0·3	+7·4	+15·7	+0·1	+3
(8) Glucose	−0·3	+6·4	+13·7	+0·1	+3
(6) Fructose	−0·3	+8·9	+14·7	0	+3
(4) Galactose	−0·1	+157·8	+13·7	0	+2
(5) Xylose	−0·2	+301·5	+12·7	+0·2	+1
(7) Arabinose	−0·3	+180·0	+18·5	+0·2	+1

* Numbers in parentheses beside infusate indicate the order of infusion in each cow.
† Determined by eye on a scale of 0–3 (see p. 694).
‡ Difference between 0 h and 7 h. The infusion was interrupted between 7 h and 8 h.
ND, not determined.

(Table 2). Results with fructose were similar. In contrast, the concentrations of TRS rose markedly when galactose, xylose and arabinose were infused. The concentration of these monosaccharides increased somewhat irregularly during each infusion but usually approached a maximum by 2–4 h after the start and fluctuated around that level thereafter. The volume of liquid in the rumen of each cow was about 80 l. Thus the total amount of carbohydrate in the rumen at the end of each infusion was 50–250 g when galactose, xylose and arabinose were infused and only 2–12 g when glucose and fructose were infused.

Table 3. *Expt 1: molar proportions of volatile fatty acids in the rumen of both cows after 8 h of infusion*

(Each carbohydrate was infused at 200 g/h for 8 h)

	Molar %					
Infusate	Acetic	Propionic	Iso-butyric	n-Butyric	Iso-valeric	n-Valeric
			Cow Adelaide			
Water	70·7	17·8	1·1	9·0	1·1	0·3
Water	71·7	17·8	0·6	7·8	1·4	0·7
Glucose	55·7	20·9	0·5	21·4	0·7	0·8
Glucose	55·9	21·7	0·6	20·6	0·9	1·3
Glucose	55·7	19·1	0·6	22·6	1·0	1·0
Fructose	58·0	19·6	0·7	20·3	0·7	0·7
Galactose	64·7	18·4	0·6	15·2	0·5	0·6
Xylose*	64·8	24·8	0·6	8·8	0·6	0·4
Arabinose	65·0	24·3	0·5	9·3	0·5	0·4
			Cow Desmine			
Water	72·1	15·8	1·4	8·8	1·4	0·5
Water	71·5	16·2	1·1	8·6	2·0	0·6
Glucose	58·9	23·8	0·7	15·1	0·8	0·7
Glucose	58·6	22·1	0·6	16·9	0·9	0·9
Glucose	57·9	23·6	0·5	16·2	0·8	1·0
Fructose	59·6	23·6	0·7	14·6	0·8	0·7
Galactose	68·2	14·0	0·9	15·6	0·5	0·8
Xylose	68·6	20·8	0·5	8·9	0·6	0·6
Arabinose	68·3	21·1	0·4	9·1	0·6	0·5

* Proportions 1 h before the planned end of the infusion; the infusion was interrupted during the last hour.

Products of fermentation. The infusion of all the carbohydrates caused increases in the concentration of VFA and decreases in pH (Table 2), clearly indicating that the disappearance of carbohydrates reflected in large measure their fermentation rather than absorption or passage out of the reticulo-rumen. The maximum falls in pH were of similar size to those following the evening feed. The concentration of VFA usually became stabilized by about 4 h after the start of each infusion in Adelaide, but with Desmine the concentration usually continued to increase throughout, though at a much reduced rate from 6 to 8 h. The size of the increase in concentration of VFA bore no obvious relationship to the amount of carbohydrate remaining in the rumen at the end of the infusion; an inverse relationship might have been expected.

The concentration of lactic acid was negligible except on one occasion following the

infusion of fructose, and even then the concentration did not approach that associated with diets containing large amounts of starch. Only glucose and fructose caused large increases in the amounts of iodophilic substances in the rumen contents (Table 2).

Proportions of VFA. With all infusions, the rate of change in the molar proportions of VFA was greatest in the first 4 h and decreased thereafter, but almost never became zero. A fall in the proportion of acetic acid was caused by all the monosaccharides, but was greatest with glucose and fructose (Table 3). These two carbohydrates also caused increases in the proportions of both propionic and n-butyric acids, though the relative amounts of the increases differed consistently between cows. Galactose caused an increase in n-butyric acid only and the pentoses increases in propionic acid only.

Expt 2. Level of glucose in vivo

It was not clear from the results of Expt 1 to what extent the differences among the carbohydrates in the proportions of VFA in the rumen at the end of the infusion reflected differences in the molar proportions of VFA produced and to what extent

Table 4. *Expt 2: mean and range of the pH and the concentrations and molar proportions of volatile fatty acids (VFA)* in the rumen of both cows before the start of infusions*

	pH	VFA (m-moles/l.)	Molar %			
			Acetic	Propionic	Iso-butyric	n-Butyric
			Cow Adelaide			
Mean	6·3	66·7	70·4	18·8	0·7	8·6
Range	6·3–6·4	62·0–69·7	69·5–71·5	18·1–19·3	0·6–0·9	7·9–9·0
			Cow Desmine			
Mean	6·6	58·9	70·3	18·7	0·9	8·3
Range	6·5–6·7	55·4–64·1	69·3–72·0	18·4–19·1	0·7–1·1	7·0–8·9

* The molar proportions of iso- and n-valeric acids were assumed to total 1·5 % for Adelaide and 1·8 % for Desmine (see p. 691).

they reflected the different rates at which the carbohydrates were fermented. The purpose of Expt 2 was to examine this problem by infusing glucose at several concentrations.

The conditions in the rumen at the start of the infusions (Table 4) and changes in concentrations and proportions of VFA caused by infusing glucose at 0 or 200 g/h (Tables 5, 6) were very similar in Expts 1 and 2. Changes in pH, in concentrations of VFA and in molar proportions of acetic acid were almost linearly related to the level of glucose infused, whereas changes in the proportions of propionic and n-butyric acids showed no clear pattern.

One difference between Expts 1 and 2 was a marked increase in the concentration of lactic acid at the two higher levels of infusion in the second experiment. This was probably related to the smaller amounts of the basal ration given in the second experiment. If the amount of glucose infused is plotted (Fig. 1) against the change in concentration of VFA plus lactic acid, an almost linear relationship results whereas, if it is

plotted against the change in concentration of VFA alone, a marked deviation from linearity occurs at the higher levels of infusion. This finding supports the general belief that when large amounts of soluble carbohydrate are fermented some of the carbohydrate is fermented to lactic acid instead of to VFA. Subsequently most of the lactic acid is probably further metabolized to VFA.

Table 5. *Expt 2: difference between rumen samples taken at the start and after 8 h of infusion in pH and concentrations of total reducing substances (TRS), volatile fatty acids (VFA) and lactic acid in both cows when glucose was infused at different concentrations*

Amount of glucose infused* (g/h)	pH	TRS (mg/100 ml)	VFA (m-moles/l.)	Lactic acid (m-moles/l.)
		Cow Adelaide		
(1) 0	+0·3	−0·3	−11·8	ND
(4) 35	+0·2	−0·5	−5·1	0·0
(3) 90	−0·1	+0·6	+3·1	0·0
(5) 145	−0·4	+0·6	+14·4	+1·4
(2) 200	−0·7	+6·1	+19·0	+7·3
		Cow Desmine		
(1) 0	+0·3	−0·3	−15·9	ND
(4) 35	0·0	−0·1	−2·5	0·0
(2) 90	0·0	+0·4	+4·6	0·0
(5) 145	−0·3	−0·2	+15·3	+2·4
(3) 200	−0·7	+15·3	+20·5	+6·5

* Numbers in parentheses beside infusate indicate the order of infusion in each cow. All concentrations of glucose were given in 2 l. water/h.
ND, not determined.

Table 6. *Expt 2: molar proportions* of volatile fatty acids in the rumen of both cows at the end of each infusion when glucose was infused at different concentrations*

Amount of glucose infused (g/h)	Molar %			
	Acetic	Propionic	Iso-butyric	n-Butyric
		Cow Adelaide		
0	71·0	18·8	1·0	7·4
35	66·6	18·7	0·6	12·3
90	63·7	18·6	0·6	15·3
145	59·1	22·9	0·6	15·6
200	55·6	22·9	0·5	19·2
		Cow Desmine		
0	73·0	17·9	1·1	6·1
35	67·5	19·5	0·8	10·3
90	63·6	21·0	0·7	12·8
145	61·1	20·9	0·6	15·5
200	57·2	25·9	0·8	14·2

* The molar proportions of iso-valeric and n-valeric acids were assumed to total 1·8% for Adelaide and 1·9% for Desmine (see p. 691).

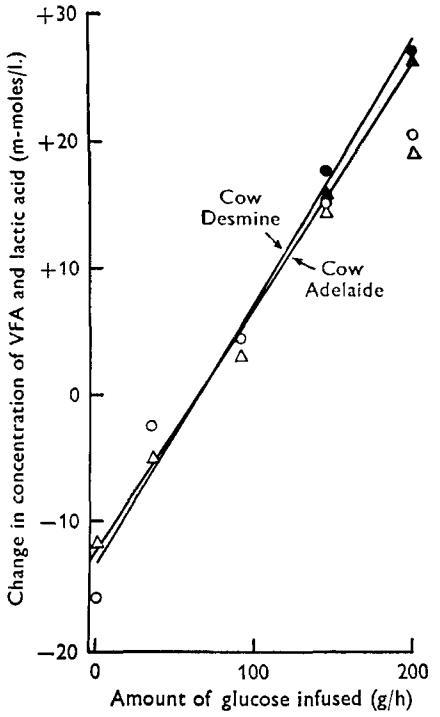

Fig. 1. Expt 2. Relation between the amount of glucose infused and the change in concentration of volatile fatty acids (VFA) (○, △) or VFA plus lactic acid (●, ▲) in the rumen during the infusion in two cows, Desmine (○, ●) and Adelaide (△, ▲). The calculated regression between the amount of glucose infused and the change in concentration of VFA plus lactic acid is shown for both cows. All infusions lasted for 8 h.

Table 7. *Expts 3 and 4: mean pH and concentration and molar proportions of volatile fatty acids (VFA) at the start of incubations in the rumen of both cows*

			Molar %					
Cow	pH	VFA (m-moles/l.)	Acetic	Propionic	Iso-butyric	n-Butyric	Iso-valeric	n-Valeric
			Expt 3					
Adelaide	6·25	70·1	71·4	19·2	0·7	7·6	0·8	0·3
Desmine	6·70	68·4	70·5	18·3	0·9	8·4	1·2	0·7
			Expt 4					
Adelaide	6·60	68·2	73·6	17·9	0·6	7·0	0·7	0·2
Desmine	6·55	62·7	73·4	17·4	0·6	7·3	1·0	0·3

Expt 3. Different carbohydrates in vitro

The conditions in the rumen contents at the start of the incubations (Table 7) were very similar to those found at the start of the in vivo experiments. The percentage of added carbohydrates that was fermented is presented in Table 8. The amounts of VFA and lactic acid produced and the pH at the end of the incubations are shown in Tables 9 and 10.

The carbohydrates could be divided into three groups on the basis of their fermentation. The first group, consisting of glucose and fructose, was almost completely fermented and produced more lactic acid, more VFA and, of the VFA, a smaller proportion of acetic acid than all the other carbohydrates. The difference between

Table 8. *Expt 3: mean percentage of carbohydrate fermented during 2 h incubations with rumen contents from both cows*

(All the carbohydrates were added at 375 mg/h. Further details of the incubation procedures are given on p. 691. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

	Carbohydrate fermented (%)	
Substrate	Cow Adelaide	Cow Desmine
Glucose	99·2	100·0
Fructose	99·7	100·0
Galactose	55·0	51·9
Xylose	52·4	46·8
Arabinose	50·5	46·5
5 % LSD	1·6	7·8

Table 9. *Expt 3: mean pH, gross production of volatile fatty acids (VFA) and molar proportions of VFA produced during the in vitro incubations with rumen contents from both cows*

(All carbohydrates were added at 375 mg/h. Further details of the incubation procedures are given on p. 691. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

			Molar %		
Substrate	pH	Total VFA (m-moles)	Acetic	Propionic	n-Butyric
		Cow Adelaide			
Glucose	5·91	4·00	53·9	30·5	15·6
Fructose	5·91	4·11	55·2	27·5	17·3
Galactose	6·13	3·30	64·9	21·2	13·9
Xylose	6·14	3·41	66·2	26·5	7·3
Arabinose	6·13	3·28	63·4	27·2	9·4
Control	6·33	1·60	69·6	22·6	7·8
5 % LSD	—	—	3·3	2·6	3·5
		Cow Desmine			
Glucose	6·16	4·03	49·9	31·5	18·6
Fructose	6·15	4·23	52·4	29·0	18·6
Galactose	6·28	3·03	64·6	18·0	17·4
Xylose	6·31	3·06	65·8	25·4	8·8
Arabinose	6·30	3·02	67·1	24·2	8·7
Control	6·53	1·30	66·4	23·3	10·4
5 % LSD	—	—	7·4	5·3	3·2

fructose and all the other carbohydrates in the proportion of acetic acid produced failed to reach significance when calculated on a net basis for cow Desmine but was significant when results for both cows were averaged. The proportion of propionic acid produced from glucose tended to be greater than that produced from fructose, but this difference was significant for cow Adelaide only. In the in vivo experiments,

when glucose and fructose were infused there was a difference between cows in the proportions of propionic and n-butyric acids in the rumen at the end of the infusion. This difference was not detected in vitro.

Table 10. *Expt 3: mean net production of volatile fatty acids (VFA) and lactic acid and molar proportions of VFA produced during the in vitro incubations with rumen contents from both cows*

(All carbohydrates were added at 375 mg/h. Further details of the incubation procedures are given on p. 691. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

Substrate	Lactic acid (m-moles)	Total VFA (m-moles)	Molar %		
			Acetic	Propionic	n-Butyric
		Cow Adelaide			
Glucose	0·77	2·40	42·3	36·4	21·3
Fructose	1·08	2·51	45·1	31·2	23·7
Galactose	0·00	1·70	58·5	21·1	20·4
Xylose	0·00	1·81	63·9	29·2	6·9
Arabinose	0·00	1·68	58·2	30·8	11·0
5 % LSD	—	—	6·4	4·4	6·6
		Cow Desmine			
Glucose	0·40	2·73	42·3	35·1	22·6
Fructose	0·60	2·93	46·4	31·4	22·2
Galactose	0·00	1·72	63·5	13·7	22·8
Xylose	0·02	1·76	65·3	27·2	7·5
Arabinose	0·01	1·71	67·5	25·1	7·4
5 % LSD	—	—	17·4	10·4	8·6

Table 11. *Expt 4: mean percentage of carbohydrate fermented during the in vitro incubations with rumen contents from both cows*

(Details of the incubation procedures are given on p. 691 The least significant difference (LSD) ($P = 0.05$) among substrates is given)

Substrate	Amount added (mg/2 h)	Carbohydrate fermented (%)	
		Cow Adelaide	Cow Desmine
Glucose	750	100·0	98·7
Sucrose	713	100·0	98·0
Glucose	375	100·0	100·0
Galactose	375	81·9	70·3
Xylose	375	88·7	71·6
5 % LSD	—	0·8	3·2

The fermentation of the other carbohydrates, galactose and the pentoses, had features in common. They were all metabolized at about half the rate of glucose and fructose though the rate of fermentation of galactose was significantly greater than that of the pentoses for Adelaide and for the mean results for both cows. They produced less VFA than glucose and fructose and virtually no lactic acid. Of the VFA produced, acetic acid constituted 56–68% on both net and gross production bases with no significant differences among the carbohydrates in this respect. Despite these similarities, the fermentation of galactose was clearly distinguished from that of the

pentoses. Galactose produced a significantly smaller proportion of propionic acid than all the other carbohydrates, whereas the pentoses produced a significantly smaller proportion of n-butyric acid than all the others.

Expt 4. Level of carbohydrate in vitro

The purposes of Expt 4 were to examine the fermentation of glucose, galactose and xylose added at half the rate used in Expt 3 and to compare the fermentation of glucose and sucrose added at the normal rate. The extent of fermentation of the carbohydrates is shown in Table 11 and the amounts of VFA and lactic acid produced and the pH in the rumen contents at the end of the incubations are shown in Tables 12 and 13. There was a slightly greater proportion of acetic acid and smaller proportion of propionic acid at the start of the incubations in Expt 4 than in Expt 3 (Table 7).

Table 12. *Expt 4: mean pH, gross production of volatile fatty acids (VFA) and proportions of VFA produced during the in vitro incubations with rumen contents from both cows*

(Details of incubation procedures are given on p. 691. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

Substrate	Amount added (mg/2 h)	pH	Total VFA (m-moles)	Molar %		
				Acetic	Propionic	n-Butyric
			Cow Adelaide			
Glucose	750	6·20	4·56	57·4	30·1	12·5
Sucrose	713	6·23	4·47	63·3	23·7	13·0
Glucose	375	6·30	3·15	59·2	27·6	13·2
Galactose	375	6·30	3·11	72·0	18·0	10·0
Xylose	375	6·28	3·49	76·7	19·6	3·7
Control	—	6·60	1·83	78·5	20·7	0·8
5 % LSD	—	—	—	5·5	3·7	2·7
			Cow Desmine			
Glucose	750	6·23	3·95	54·0	32·6	13·4
Sucrose	713	6·25	4·01	59·0	27·9	13·1
Glucose	375	6·30	2·67	51·2	31·6	17·2
Galactose	375	6·30	2·47	67·6	18·0	14·4
Xylose	375	6·33	2·52	71·4	22·0	6·6
Control	—	6·55	1·17	73·3	21·8	4·9
5 % LSD	—	—	—	4·1	2·4	3·0

Sucrose did not differ markedly from glucose in the extent of its fermentation or the amounts of VFA produced. However, it did differ in producing less lactic acid, a significantly greater proportion of acetic acid and a significantly smaller proportion of propionic acid.

One effect of adding glucose, galactose and xylose at half the level used in Expt 3 was to cause smaller differences in products and so a relatively greater variability in results due to analytical and other errors. Glucose provided the only direct comparison of the effect of different levels of substrate within one experiment. There was good agreement between the effects of the two levels of glucose on proportions of VFA produced when these were calculated from gross production, but significant dif-

ferences were detected on a net production basis. Comparison of the products of fermentation from galactose and xylose at the two levels of addition could only be made between experiments and was also inconclusive. Although the results were inadequate to show whether the products of fermentation were the same at the two levels of addition, they did confirm in general the relative differences among the carbohydrates found at the higher level of addition in terms of both rates of fermentation and the proportions of VFA produced.

Table 13. *Expt 4: mean net production of volatile fatty acids (VFA) and lactic acid and molar proportions of VFA produced during the in vitro incubations with rumen contents from both cows*

(Details of the incubation procedures are given on p. 691.
The least significant difference (LSD) ($P = 0.05$) among substrates is given)

Substrate	Amount added (mg/2 h)	Lactic acid (m-moles)	Total VFA (m-moles)	Molar %		
				Acetic	Propionic	n-Butyric
			Cow Adelaide			
Glucose	750	0·35	2·73	42·0	36·8	21·2
Sucrose	713	0·00	2·64	51·6	26·1	22·3
Glucose	375	0·00	1·32	29·9	37·9	32·3
Galactose	375	0·00	1·28	65·6	13·3	21·1
Xylose	375	0·00	1·66	76·8	17·7	5·5
5 % LSD	—	—	—	6·3	5·8	4·5
			Cow Desmine			
Glucose	750	0·70	2·78	47·0	36·9	16·1
Sucrose	713	0·17	2·84	54·0	30·3	15·7
Glucose	375	0·00	1·50	35·7	38·9	25·4
Galactose	375	0·00	1·30	60·0	15·7	24·3
Xylose	375	0·00	1·35	67·3	23·4	9·3
5 % LSD	—	—	—	4·4	4·6	3·3

Expt 5. Extended incubation in vitro

During the first 2 h when substrates were being added the mean gross production of VFA in the glucose, xylose and control flasks was 3·29, 3·46 and 1·64 m-moles respectively. In the second 2 h period when no further substrate was added, 1·89, 1·81 and 1·90 m-moles VFA respectively were produced. Hence there was no evidence that polysaccharide, that might have been formed in the presence of added substrate in the first 2 h, was metabolized to VFA in the succeeding 2 h.

DISCUSSION

Validity of techniques

In earlier experiments (Phillipson & McAnally, 1942; Elsden, 1945; Waldo & Schultz, 1956; Hueter *et al.* 1958) designed to examine the metabolism of simple carbohydrates, all the substrate has been introduced into the rumen over a very short period, usually by direct addition through a fistula. A very rapid but brief burst of fermentation ensues and responses are difficult to measure accurately or to interpret. The intraruminal infusion used in the present in vivo experiments provided a con-

tinuous source of substrate for several hours. It was hoped that by this means a steady-state situation would be approached. The steady state was defined for this purpose as a period of at least 2 h during which changes in the concentration of fermentation products and of any unfermented carbohydrate approached zero. To increase the sensitivity of the technique, infusion of substrates did not begin until about 14 h after the one daily feed. By this time the rate of fermentation of the feed, and hence changes in the concentration of total VFA, were approaching the daily minimum, and changes in the proportions of VFA were very small.

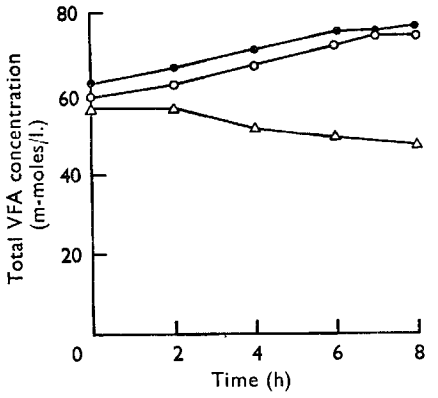

Fig. 2. Expt 1. Concentration of volatile fatty acids (VFA) in the rumen during the infusion of glucose (○), galactose (●) or water (△) into the rumen of cow Desmine for 8 h. Carbohydrates were infused at 200 g/h; water and carbohydrate solutions were infused at 2 l./h.

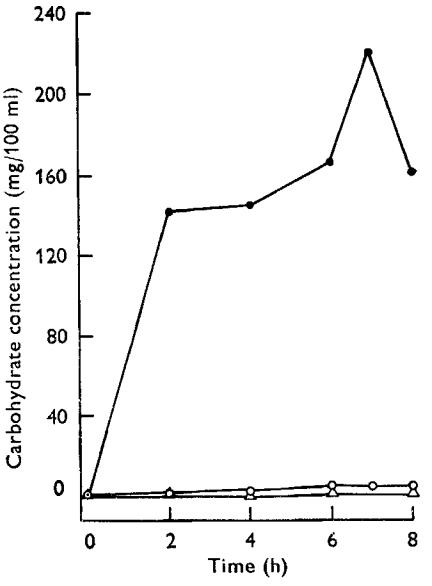

Fig. 3. Expt 1. Concentration of carbohydrate in the rumen during the infusion of glucose (○), galactose (●) or water (△) into the rumen of cow Desmine for 8 h. Carbohydrates were infused at 200 g/h; water and carbohydrate solutions were infused at 2 l./h.

The changes in cow Desmine illustrated in Figs. 2–4 show that a steady-state situation was approached, though at no time did the changes in concentrations of metabolites reach zero. More satisfactory results were obtained with cow Adelaide. The highly reproducible response to repeated glucose infusions (Tables 2, 3) illustrates the sensitivity of the technique.

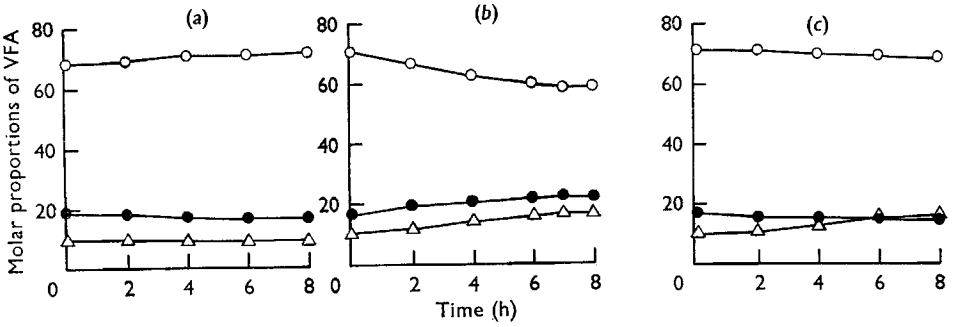

Fig. 4. Expt 1. Molar proportions of acetic (○), propionic (●) and n-butyric (△) acids in the rumen during the infusion of (*a*) water, (*b*) glucose or (*c*) galactose into the rumen of cow Desmine for 8 h. Carbohydrates were infused at 200 g/h; water and carbohydrate solutions were infused at 2 l./h.

The in vitro studies were intended to provide evidence regarding the absolute amounts of carbohydrate metabolized and of fermentation products produced. Such evidence could not be obtained with the in vivo technique. The validity of in vitro techniques has been discussed at length elsewhere (Warner, 1956; Hungate, 1966). In general, the shorter the period of incubation the greater is likely to be the validity of the results, but the ultimate criterion must be the degree of agreement between results of in vivo and in vitro experiments. In preliminary studies the criterion chosen for deciding that fermentation approached that obtained in vivo was that glucose, when added in vitro at a rate equivalent to that used in vivo, should not accumulate during the 2 h incubation. The main factor affecting this was the presence of solid material from the rumen contents; in rumen liquor filtered through two layers of surgical gauze, the amount of glucose metabolized was only about 75% of that in whole rumen contents. Phillipson & McAnally (1942) reported that the supernatant liquid from centrifuged rumen contents metabolized glucose and lactic acid less rapidly than fluid from which solid had been removed by filtration through muslin. Presumably these procedures effect removal of protozoa and bacteria to varying degrees and hence alter fermentation rates.

Although the results of the in vivo and in vitro techniques cannot be compared in absolute terms, the close similarity of the relative differences among the carbohydrates as determined by the two techniques provides good evidence of the validity of the in vitro procedure. Further confirmation will require development of accurate techniques to determine products of fermentation in vivo.

Fermentation of carbohydrates

Since results from the in vivo and in vitro experiments were in such close agreement, they will be considered together. The experiments have shown quite clearly that such chemically similar substrates as soluble carbohydrates can be metabolized by a mixed population of rumen bacteria and protozoa at rates that differ by a factor of at least two and that they can produce end-products of fermentation in quite different proportions. Such differences have been shown previously, but few experiments have involved comparison of in vivo and in vitro techniques and none has provided the degree of control achieved in the present studies.

On the basis of rates of fermentation, the carbohydrates can be divided into two groups: glucose, fructose and sucrose that were rapidly fermented, and galactose, xylose and arabinose that were fermented at only half the rate of the others. The studies of Phillipson & McAnally (1942) clearly showed that galactose, lactose and maltose were fermented less rapidly than sucrose, glucose and fructose. The fermentation of pentoses has been examined in detail by several workers. Howard (1958) showed that D-xylose and L-arabinose were the most rapidly fermented of several pentoses studied, and McNaught (1951) reported that these two pentoses supported microbial growth as well as sucrose, galactose and fructose. However, neither these nor other workers appear to have compared the rate of fermentation of pentoses with that of hexoses.

Examination of the products of fermentation permits further subdivision of the carbohydrates. Of the rapidly metabolized carbohydrates, glucose and fructose were metabolized to similar end-products though small differences were detected in the amounts of lactic acid and the molar proportion of propionic acid produced. Sucrose was examined in less detail and only in vitro, but the results, which have been largely confirmed by later more extensive experiments (Sutton, 1967, unpublished), indicated that, compared with glucose and fructose, sucrose produced less lactic acid and, of the VFA, a greater proportion of acetic acid and a smaller proportion of propionic acid.

The more slowly metabolized carbohydrates all produced a relatively larger proportion of acetic acid than the other three sugars. However, galactose and the pentoses, although producing similar proportions of acetic acid, were clearly differentiated from one another by the relative proportions of propionic and n-butyric acids produced.

The relationship in the present experiments between rate of fermentation of the carbohydrates and the proportion of acetic acid produced does not appear to be a causal one. In Fig. 5, results with cow Desmine from Expts 1 and 2 have been combined. For a given change in concentration of total VFA resulting from the infusions, a smaller decrease in the proportion of acetic acid in the rumen occurred when galactose, xylose or arabinose was infused than when water, glucose at various levels, or fructose was infused. In the in vitro studies glucose was added at only half the standard rate in Expt 4; its rate of fermentation thus approximated to that of the more slowly metabolized carbohydrates in Expt 3, but it still produced a much smaller proportion of acetic acid than the other carbohydrates. Thus evidence is strong that

the differences among the carbohydrates in the proportions of VFA produced by fermentation are not caused by the different rates at which they are fermented.

Information from other sources regarding proportions of VFA produced from soluble carbohydrates is contradictory and provides no clear picture. The results of in vitro (Elsden, 1945; Hershberger, Bentley, Cline & Tyznik, 1956; Hueter *et al.* 1958) and in vivo (Elsden, 1945; Waldo & Schultz, 1956; Hueter *et al.* 1958) experiments clearly indicate that glucose produces a relatively small proportion of acetic

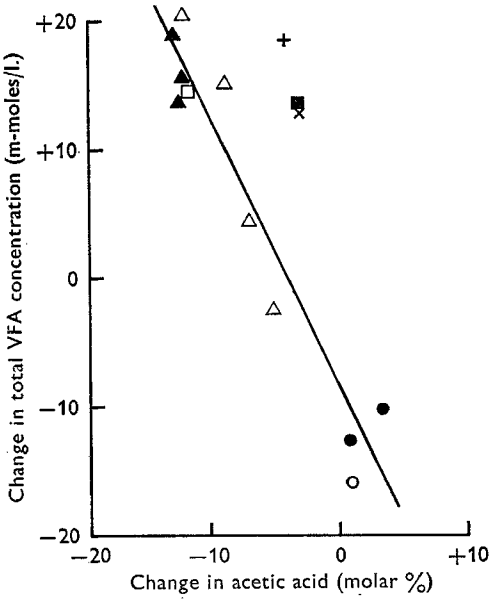

Fig. 5. Expts 1 and 2. Relation between the change in concentration of total volatile fatty acids (VFA) and the change in the molar proportion of acetic acid in the rumen when various substrates were infused for 8 h into the rumen of cow Desmine. Solutions infused were water (Expt 2, ○; Expt 1, ●), glucose (Expt 2, △; Expt 1, ▲), fructose (□), galactose (■), xylose (×), and arabinose (+); all were infused at 2 l./h. In Expt 1 all carbohydrates were infused at 200 g/h; in Expt 2 glucose was infused at 35, 90, 145 and 200 g/h.

acid, but there is disagreement about the relative proportions of propionic and butyric acid produced. Some disagreement was apparent in the present studies in that glucose consistently caused a smaller increase in the proportion of propionic acid in Adelaide than in Desmine in the in vivo studies but not in vitro. There appears to be no previous information concerning the VFA produced from galactose. Much more exists regarding the fermentation of the pentoses (McNaught, 1951; Heald, 1952; Doetsch *et al.* 1953) but there is little agreement although the results of McNaught (1951), who used an in vitro technique similar to our own, agree with ours in showing that little butyric acid was produced from either D-xylose or L-arabinose.

Of other products of fermentation, lactic acid usually appears as an intermediate product when glucose is given at a sufficiently high level (Phillipson & McAnally, 1942; Elsden, 1945). Comparison of the results of Expts 1 and 2 strongly suggests that the accumulation of lactic acid in the rumen depends not only on the amount of glucose added but also on the amount of basal ration consumed. The level of feeding

used during the in vitro studies was the same as that used in Expt 2. Hence the appearance of lactic acid as an important product of glucose fermentation in vitro is in agreement with the in vivo results.

In the in vitro experiment reported here, lactic acid was produced from fructose and, to a much lesser extent, from sucrose as well as from glucose; virtually none was produced from galactose, xylose or arabinose. These differences among the carbohydrates agree with earlier results (Phillipson & McAnally 1942; McNaught, 1951; Heald, 1952; Robinson *et al.* 1955) and probably reflect largely the different rates at which they are fermented although the differences between glucose, fructose and sucrose in the amounts of lactic acid produced suggest that rate of fermentation is not the only factor involved.

The detection of storage polysaccharide in our experiments relied on the formation of a blue colour when iodine was added. Only samples from in vivo experiments were examined. Glucose and fructose produced large amounts of iodophilic substance, galactose less and the pentoses very little. There is considerable evidence that pentoses as well as hexoses can be metabolized to bacterial polysaccharide by mixed rumen bacteria in vitro (McNaught, 1951; Doetsch *et al.* 1953; Howard, 1958) though there is little information from in vivo work. Among variables that have been shown to affect the extent of such polysaccharide formation are species of bacteria (Hobson & Mann, 1955; Doetsch, Howard, Mann & Oxford, 1957) and the amount of carbohydrate available (Robinson *et al.* 1955). The differences among the carbohydrates detected in the present experiments may reflect primarily the differences in their rates of metabolism.

It seems probable that the main reason that such chemically similar substrates as soluble carbohydrates are fermented at different rates and to different proportions of products is because they are fermented by different bacteria or protozoa. This is particularly borne out by the differences between glucose, fructose and sucrose. Fermentation of sucrose is almost inevitably preceded by hydrolysis to the constituent monosaccharides. If these monosaccharides were then released generally into the rumen fluid, it would be reasonable to expect them to be metabolized as are the infused monosaccharides. If, however, only some of the bacteria that can ferment glucose and fructose can also hydrolyse sucrose, then quite different populations of bacteria could be involved in the fermentation of these three substrates particularly if the hydrolysis occurs intracellularly or in close proximity to the cell so that the released monomers do not become available to the general rumen microflora and fauna. Hungate (1966), listing the fermentation of substrates by twenty-two different bacteria, reports several species that can ferment one or two, but not all of the carbohydrates, glucose, fructose and sucrose. Similarly the numbers that can ferment galactose (11), xylose (7) or arabinose (10) are considerably fewer than those able to ferment glucose (18), fructose (14) or sucrose (15).

Quantitative aspects of VFA production

In Tables 14 and 15, the recovery of fermented carbohydrate is presented. The calculations have been based on carbon as a common denominator. These quantitative

aspects of the fermentation can be calculated only from the in vitro experiments. How far these results apply to the in vivo situation cannot be determined by the present experiments although the similarity of the results from the two techniques in relative terms is encouraging. A second problem is that results are insufficient to indicate whether gross or net production of VFA is the more appropriate figure. This depends on whether the fermentation which occurred in the control flasks continued at the same rate in the presence of added carbohydrates, in which event net production

Table 14. *Expt 3: percentage recovery of fermented carbohydrates in volatile fatty acids (VFA) and lactic acid following in vitro incubation with rumen contents from both cows.*

(All carbohydrates were added at 375 mg/h. All results are calculated on a carbon basis. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

	Recovery				
		VFA		VFA + lactic acid	
Substrate	Lactic acid	Net	Gross	Net	Gross
		Cow Adelaide			
Glucose	9·3	27·0	42·3	36·3	51·6
Fructose	13·0	28·0	43·2	41·0	56·3
Galactose	0·0	32·1	59·7	32·1	59·7
Xylose	0·0	33·6	62·9	33·6	62·9
Arabinose	0·0	33·5	63·9	33·5	63·9
5% LSD	2·3*	3·5	3·5	4·6	3·9
		Cow Desmine			
Glucose	4·8	30·6	43·4	35·4	48·2
Fructose	7·2	32·3	45·1	39·5	52·3
Galactose	0·0	34·5	59·1	34·5	59·1
Xylose	0·0	36·6	63·7	36·6	63·7
Arabinose	0·0	36·3	63·5	36·3	63·5
5% LSD	1·8*	8·2	11·1	5·2	7·7

* Calculated from the analysis of variance for glucose and fructose only, since only traces of lactic acid were produced from the pentoses and none from galactose.

would be the true figure, or whether it was partially or completely inhibited, in which event gross production or some intermediate value would be more correct. The only evidence bearing on this point from the present experiments comes from a comparison of carbon recovery from carbohydrates added at two different levels (Expts 3 and 4). Agreement was better when calculations were based on net recovery than when they were based on gross recovery. This suggests that net conversion may be the more correct figure, but even this conclusion depends on the assumption that there is no difference in the proportions of other products of fermentation, such as starch, at the two levels of addition.

In Expt 3 (Table 14) recovery of metabolized carbohydrates in VFA and lactic acid varied from 32% to 41% on a net basis and from 48% to 64% on a gross basis. Differences among the carbohydrates varied according to whether they were compared on a net or gross recovery basis. Of the net recovery in VFA and lactic acid, about

25% of the fructose and 20% of the glucose were in lactic acid. Recovery of glucose in lactic acid was significantly less than that of fructose and recovery of glucose in VFA plus lactic acid also tended to be less, though this was not always significant. In Expt 4 (Table 15) recoveries were in a similar range, although gross recovery tended to be greater for the carbohydrates added at half the standard level. Recovery of metabolized sucrose in lactic acid and VFA plus lactic acid was significantly less than recovery of metabolized glucose. The net recovery of metabolized carbohydrate in VFA and lactic acid was low when considered in terms of the economy of utilization of soluble carbohydrates by the ruminant yet it is supported by other in vitro studies.

Table 15. *Expt 4: percentage recovery of fermented carbohydrates in volatile fatty acids (VFA) and lactic acid following in vitro incubation with rumen contents from both cows*

(All results are calculated on a carbon basis. The least significant difference (LSD) ($P = 0.05$) among substrates is given)

Substrate	Amount added (mg/2 h)	Recovery: Lactic acid	Recovery: VFA Net	Recovery: VFA Gross	Recovery: VFA + lactic acid Net	Recovery: VFA + lactic acid Gross
Cow Adelaide						
Glucose	750	4·2	30·5	46·5	34·7	50·7
Sucrose	713	0·0	28·6	44·6	28·6	44·6
Glucose	375	0·0	31·9	64·0	31·9	64·0
Galactose	375	0·0	32·0	72·3	32·0	72·3
Xylose	375	0·0	34·3	71·5	34·3	71·5
5% LSD	—	0·0*	1·4	1·6	1·4	5·6
Cow Desmine						
Glucose	750	8·5	30·3	41·5	38·8	50·0
Sucrose	713	2·0	30·3	41·6	32·3	43·6
Glucose	375	0·0	34·6	56·7	34·6	56·7
Galactose	375	0·0	39·1	69·4	39·1	69·4
Xylose	375	0·0	36·5	66·1	36·5	66·1
5% LSD	—	2·9*	2·2	2·7	2·5	1·5

* Calculated from the analysis of variance for glucose and sucrose added at the higher level only. No lactic acid was produced from the other substrates.

McNaught (1951) reported the recovery of 41–52% of the carbon from xylose and arabinose in VFA; this is rather higher than our net recovery values but she used a longer period of incubation and did not correct for production in control flasks, which she described as very low. Heald (1952) reported the recovery of 36–56% of the carbon from xylose in VFA; if corrected for production of VFA in control flasks, recovery would be reduced to approximately 29–45%. Howard (1958) reported that only 30% of the D-xylose and L-arabinose metabolized was converted into VFA in the early hours of a 24 h incubation.

If it is accepted that only between 30% and 45% of the metabolized carbohydrate is converted into VFA and lactic acid, it becomes important to consider what may be the other products of fermentation. Information from various sources (McNaught, 1951; Howard, 1958; Thomas, 1960; Hungate, 1966) suggests that 30–45% of the

carbon from metabolized carbohydrate is converted into VFA and lactic acid, about 30% into microbial polysaccharide, 10–13% into microbial protein and 11–14% into carbon dioxide and methane. As has been discussed by McNaught (1951), considerable error is attached to many of these values.

The fate of the large amount of bacterial polysaccharide calculated to be formed is not clear, though it may be of considerable significance to the carbohydrate economy of the animal. In Expt 5 we found no evidence of appreciable production of VFA from storage polysaccharide when the incubation was continued for 2 h after the last addition of glucose or xylose but Thomas (1960) showed that storage polysaccharide was gradually metabolized during a 24 h incubation. Whether the starch becomes available for fermentation to VFA in the rumen would appear to depend on whether the rate of passage of bacteria and protozoa out of the rumen exceeds the rate at which the starch can be metabolized.

Relevance to fermentation of feeds

The significance of the results obtained in the present experiments in relation to the fermentation of normal feeds in ruminants depends on various factors, of which the availability of the substrates is dominant. Natural feeds usually contain only small amounts of simple monosaccharides. Fresh herbage includes free sucrose, glucose and fructose in variable amounts, the total rarely exceeding 15% of the dry matter. Certain roots contain large amounts of soluble carbohydrates; Gaillard (1958) reported that 69% of the dry matter of mangolds was sucrose. By far the largest proportion of the carbohydrates studied here is present as polymers or some other conjugated form such as galactosyl lipid (Bailey, 1962). Free glucose has been detected in the rumen during the fermentation of large amounts of starch from wheat (Ryan, 1964) or maize (Sutton, unpublished) and free xylose has also been shown to accumulate during xylan digestion (Howard, 1955; Pazur, Budovich, Shuey & Georgi, 1957), but in general evidence is conflicting regarding the extent to which breakdown of carbohydrate complexes involves the release of the free monosaccharide unit into the rumen fluid (see Hungate, 1966).

There seems little likelihood that the fermentation of polysaccharides is closely related to that of their constituent monosaccharides. Important cellulolytic and xylolytic bacteria are unable to ferment monosaccharides and many sugar-fermenting bacteria cannot attack polysaccharides. In the present experiments the fermentation of sucrose differed clearly from that of glucose and fructose, emphasizing that it is invalid to extrapolate results obtained from monosaccharides to even the simplest polymer without experimental evidence.

Another important factor is the nature of the fermentation proceeding in the rumen before the carbohydrates are added. In the present experiments cows were given a large proportion of hay. Reducing the proportion of hay or giving the carbohydrates themselves for an extended period might induce changes in the rumen fermentation. There is some evidence that such changes result in the production of different proportions of VFA from the same substrate (Eusebio, Shaw, Leffel, Lakshmanan & Doetsch, 1959; Baldwin, Wood & Emery, 1963).

The results of the present experiments have provided a firm basis for comparing the fermentation of certain simple soluble carbohydrates but any attempt at extensive extrapolation of these results, obtained under relatively simple and closely defined conditions, to other and more complex situations should be avoided until more evidence is available. The experiments have also confirmed findings of other workers that only a relatively small proportion of soluble carbohydrate is converted directly into VFA in vitro. There appears to be no evidence as to whether such low conversion rates apply to the in vivo situation; information on this point is clearly needed.

I am most grateful to Dr C. C. Balch for his advice and encouragement throughout this work and to Mr D. R. Westgarth for his assistance with many of the statistical analyses. I thank Professor J. A. F. Rook for his helpful interest, Dr A. T. Cowie for establishing the rumen fistulas, Mr V. W. Johnson for care of the experimental animals, and Mr E. Schuller and Mr K. M. T. Hearne for their skilled technical assistance. The work was supported in part by a generous grant from the Sugar Research Foundation Inc., New York.

REFERENCES

Annison, E. F. (1954). *Biochem. J.* **57**, 400.
Bailey, R. W. (1962). *Proc. N.Z. Soc. Anim. Prod.* **22**, 99.
Balch, C. C. & Cowie, A. T. (1962). *Cornell Vet.* **52**, 206.
Baldwin, R. L., Wood, W. A. & Emery, R. S. (1963). *J. Bact.* **85**, 1346.
Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants.* London: Hutchinson.
Doetsch, R. N., Howard, B. H., Mann, S. O. & Oxford, A. E. (1957). *J. gen. Microbiol.* **16**, 156.
Doetsch, R. N., Robinson, R. Q., Brown, R. E. & Shaw, J. C. (1953). *J. Dairy Sci.* **36**, 825.
Eusebio, A. N., Shaw, J. C., Leffel, E. C., Lakshmanan, S. & Doetsch, R. N. (1959). *J. Dairy Sci.* **42**, 692.
Elsden, S. R. (1945). *J. exp. Biol.* **22**, 51.
Elsden, S. R. & Gibson, Q. H. (1954). *Biochem. J.* **58**, 154.
Gaillard, B. D. E. (1958). *J. Sci. Fd Agric.* **9**, 346.
Heald, P. J. (1952). *Biochem. J.* **50**, 503.
Hershberger, T. V., Bentley, O. G., Cline, J. H. & Tyznik, W. J. (1956). *J. agric. Fd Chem.* **4**, 952.
Hobson, P. N. & Mann, S. O. (1955). *J. gen. Microbiol.* **13**, 420.
Hodson, H. H., McGilliard, A. D., Jacobson, N. L. & Allen, R. S. (1965). *J. Dairy Sci.* **48**, 1652.
Howard, B. H. (1955). *Biochem. J.* **60**, i.
Howard, B. H. (1958). *Proc. Nutr. Soc.* **17**, xxvi.
Hueter, F. G., Gibbons, R. J., Shaw, J. C. & Doetsch, R. N. (1958). *J. Dairy Sci.* **41**, 651.
Hungate, R. E. (1966). *The Rumen and Its Microbes.* London: Academic Press Inc.
McDougall, E. I. (1948). *Biochem. J.* **43**, 99.
McNaught, M. L. (1951). *Biochem. J.* **49**, 325.
Pazur, J. H., Budovich, T., Shuey, E. W. & Georgi, C. E. (1957). *Archs Biochem. Biophys.* **70**, 419.
Phillipson, A. T. & McAnally, R. A. (1942). *J. exp. Biol.* **19**, 199.
Robinson, R. Q., Doetsch, R. N., Sirotnak, F. M. & Shaw, J. C. (1955). *J. Dairy Sci.* **38**, 13.
Rook, J. A. F. & Balch, C. C. (1961). *Br. J. Nutr.* **15**, 361.
Ryan, R. K. (1964). *Am. J. vet. Res.* **25**, 653.
Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.
Storry, J. E. & Millard, D. (1965). *J. Sci. Fd. Agric.* **16**, 417.
Thomas, G. J. (1960). *J. agric. Sci., Camb.* **54**, 360.
Tilley, J. M. A., Canaway, R. J. & Terry, R. A. (1964). *Analyst, Lond.* **89**, 363.
Waldo, D. R. & Schultz, L. H. (1956). *J. Dairy Sci.* **39**, 1453.
Warner, A. C. I. (1956). *J. gen. Microbiol.* **14**, 733.

Printed in Great Britain