Utilization of ^{14}C-labelled *Bacillus subtilis* and *Escherichia coli* by sheep

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1. *Bacillus subtilis* and *Escherichia coli* were grown on ^{14}C-labelled glucose and used for the preparation of labelled whole cells, cell walls, cell contents and peptidoglycan.

2. The radioactive samples were injected into the abomasum of sheep and the ^{14}C appearing in expired air, plasma glucose, urine and faeces was determined. Whole cells were also injected into the rumen and the incorporation of ^{14}C into volatile fatty acids was measured.

3. All the bacterial preparations, including cell walls, were extensively digested and absorbed. Less than 15% of the radioactivity was recovered in the faeces.

4. Up to 20% of the radioactivity injected was recovered in expired carbon dioxide with only 2·4–8·1% passing through the glucose pool.

5. It has been calculated that under the conditions of the experiment 18·5% of the total glucose entering the body pool of glucose in 24 h was derived from bacterial carbon.

Studies with sheep given roughage diets show that the rates of entry of glucose into the body pools of glucose (glucose entry rates) are close to those found in monogastric animals in the postabsorptive state (Annison & White, 1961; Kronfeld & Simesen, 1961; Bergman, 1963; Ford, 1963) and that the glucose entry rate, based on these results, is of the order of 100 g glucose/day for a fed adult sheep.

Estimates of the quantities of reducing sugars entering the abomasum of sheep have indicated that the contribution of carbohydrates of bacterial origin to the glucose requirements is only approximately 5 g glucose/day (Heald, 1951). On this basis most of the glucose on roughage diets must arise by gluconeogenesis.

Studies using liver slices (Leng & Annison, 1963) or the intact animal (Annison, Leng, Lindsay & White, 1963) have shown that, of the major short-chain fatty acids produced in the rumen, only propionate is glucogenic. Krebs & Yoshida (1963) showed that kidney cortex of sheep also readily converts propionate into glucose. Recent measurements of propionate production rates in the rumen have indicated that the quantity of propionate produced could meet the measured glucose entry rates in sheep (Bergman, Reid, Murray, Brockway & Whitelaw, 1965; Leng & Leonard, 1965). However, only half the glucose is synthesized from propionate produced in the rumen (Leng, Steel & Luick, 1967). This suggests that protein could be an important source of glucose. As gluconeogenesis from protein could supply 50–60 g glucose/100 g protein (Krebs, 1965), it seemed logical to investigate the contribution

that bacteria could make in supplying the glucose requirements of sheep, following their digestive breakdown in the alimentary tract.

A characteristic feature of bacteria is that they contain peptidoglycan, a heterogeneous polymer responsible for the structural rigidity of the cell (Martin, 1966). As there is no information in the literature about the nutritive value of this bacterial component or about the entire bacterial cell wall, it was decided to investigate the availability of these parts of the bacterial cell to the sheep separately from the whole bacterial cell. Accordingly, ^{14}C-labelled *Bacillus subtilis* and *Escherichia coli* whole cells, cell walls and cell contents (whole cells minus cell walls) and peptidoglycan from *B. subtilis* were injected into the abomasum of the feeding sheep and the contribution to carbon dioxide in expired air and plasma glucose was studied. Labelled *B. subtilis* and *E. coli* whole cells were also injected into the rumen and their metabolism was followed.

EXPERIMENTAL

Preparation of radioactive bacterial samples

E. coli was cultured on the mineral salts medium C of Roberts, Abelson, Cowie, Bolton & Britten (1955), with 0·2% glucose as sole carbon source, and 0·05 μc [U-^{14}C]glucose per ml culture medium as described by Coleman (1967). Cells were grown at 38° for 24 h in a 10 l. batch in a large fermentor which was vigorously aerated and which was inoculated with a 1 l. culture of *E. coli* cultured on [^{14}C]glucose. Bacteria were harvested by centrifugation at 5000*g* for 15 min at 4°, and the preparation was washed twice with 0·9% (w/v) NaCl and once with water.

One-fifth of the total cells harvested were freeze-dried to be used as '*E. coli* whole cells'. The remainder were mechanically disrupted by means of the French-press. Broken cells were centrifuged at 10000*g* for 20 min and the supernatant fluid containing cell contents was freeze-dried to be used as '*E. coli* cell contents'. The pellet of cell walls was washed three times in 0·9% (w/v) NaCl and once in water, and then freeze-dried to be used as '*E. coli* cell walls'. The yield of cell walls was 16% on a dry-weight basis.

B. subtilis var. *niger* was cultured by growing it in mineral salts medium high in phosphate as described by Tempest, Dicks & Ellwood (1968), with 0·5% glucose as energy source. As for *E. coli*, *B. subtilis* was cultured on medium containing 0·05 μc [U-^{14}C]glucose per ml.

Cells were grown in two 15 l. batches as for *E. coli*, a 10% inoculum being used. The culture was harvested in a refrigerated centrifuge at 3000*g* for 10 min. In order to remove an extracellular pigment produced by this organism, the bacterial pellet was resuspended in 0·9% (w/v) NaCl and centrifuged at 2500*g* for 10 min. The bacteria were washed twice more in 0·9% (w/v) NaCl and finally with water.

One-tenth of the total bacteria harvested were freeze-dried to be used as '*B. subtilis* whole cells'. The remainder was mechanically disrupted by sonicating a thick suspension of cells in an ultrasonic disintegrator (20 kc/sec MSE Ultrasonic Disintegrator) for 4 min. Unbroken cells were removed by centrifuging at 2500*g* for 10 min, and the small pellet of whole cells was discarded. The broken cells were then

centrifuged at 15000*g* for 20 min to deposit cell walls. The supernatant fluid containing cell contents was freeze-dried to be used as '*B. subtilis* cell contents'. The pellet of cell walls was washed three times in 0·9% (w/v) NaCl and once in water. Half the washed cell walls were freeze-dried to be used as '*B. subtilis* cell walls'. The yield of cell walls was 30% on a dry-weight basis. The remaining half were used for the preparation of the peptidoglycan.

Preparation of B. subtilis *peptidoglycan*

Half the *B. subtilis* cell walls prepared as above were heated in a water bath at 100° for 15 min to inactivate autolytic enzymes. The cell walls were digested with ribonuclease, pepsin and trypsin to remove adhering protoplasm, and were then extracted three times in 10% (w/v) trichloroacetic acid at 4° for 24 h to remove carbohydrate and teichoic acids from the peptidoglycan backbone of the cell wall (Armstrong, Baddiley & Buchanan, 1960). The peptidoglycan so prepared was washed three times in water and freeze-dried to be used as '*B. subtilis* peptidoglycan'. The yield of peptidoglycan was 6% on a dry-weight basis.

Analysis of radioactive preparations

Specific activity of samples. Samples (1 mg) were dissolved in 0·5 ml of 2 M-NaOH by heating in sealed tubes at 110° for 1 h. Following this, 1·5 ml water were added to lower the concentration of alkali to 0·5 M and a 1 ml sample was added to 10 ml dioxane scintillation solution (Bray, 1960) and counted in a liquid scintillation counter (Ansitron).

Chemical analyses of samples. Samples were assayed for total carbohydrates, amino sugars and amino acids as previously described (Hoogenraad & Hird, 1970). Sugars were also determined by gas–liquid chromatography as described previously. A carbon analysis was carried out on each of the samples by the C.S.I.R.O. Microanalytical Service.

Experimental animals and feeding regime

Three merino sheep were used. Two sheep were provided with abomasal cannulas and double tracheal fistulas (Young & Webster, 1963) and the third with a rumen cannula. Sheep were housed singly in metabolism cages and were given 800 g of lucerne chaff in twenty-four equal amounts at hourly intervals from an automatic feeder (Minson & Cowper, 1966). All the animals received the experimental ration for 10 weeks before the experiments.

Measurement of gas exchange

Energy expenditure was monitored with an open circuit calorimeter. Two separate methods of collecting expired gas were used.

Method 1. This was used for two of the sheep (nos. 215 and 196); double tracheal cannulas were fitted with a tracheal flow divider (Corbett, Leng & Young, 1969), and all the expired gases were collected in a moving stream of air.

Method 2. A system, more comfortable for the animal, was used for monitoring gas

exchange by the third animal (no. C 172). The head of this sheep was confined in a perspex chamber containing water and in which the animal could be fed automatically at hourly intervals. The chamber was sealed with a plastic apron attached to the animal's neck and fitted to the sides of the chamber. Air in the chamber was continually mixed with a fan and mixed air was drawn from the chamber at a rate which maintained the CO_2 concentration in the analyser between 0·3 and 1·3% (v/v).

In both analyser systems, water vapour was removed by ice-salt and $CaCl_2$ traps and the volume of air recorded with a dry-gas meter. Samples of dry gas were withdrawn from the main air stream for continuous monitoring of oxygen with a paramagnetic oxygen analyser (Beckman Inc.) and of CO_2 with an infrared analyser (Onera 80).

A known volume of air was continuously withdrawn from the main air stream for monitoring radioactivity. The ^{14}C content of the gas was assayed with a 4·3 l. ion chamber attached to a vibrating reed electrometer (Carey Model 31) and specific radioactivity was obtained by dividing the rate of expiration of ^{14}C by the rate of expiration of CO_2.

Experimental procedure

Successive injections of the ^{14}C-labelled bacterial preparations were made with at least a 3-day interval to allow the animal to recover from all aspects of the procedure. The sheep was fitted, 2 h before an experiment, with the apparatus to monitor respiratory

Table 1. *Amount of radioactivity injected and site of injection of bacterial samples*

(At the start of the experiments sheep no. 215 weighed 42·2 kg, no. 196 weighed 33·7 kg and no. C 172 weighed 35·5 kg)

Radioactive sample	Sheep no.	Dose injected (μc)	Site of injection
Bacillus subtilis:			
Whole cells	215	30	Abomasum
Cell contents	215	30	Abomasum
Cell walls	196	30	Abomasum
Peptidoglycan	196	4·2	Abomasum
Escherichia coli:			
Whole cells	215	30	Abomasum
Cell contents	215	100	Abomasum
Cell walls	215	20	Abomasum
B. subtilis whole cells	C 172	85	Rumen
E. coli whole cells	C 172	42·6	Rumen

gas exchange and expired ^{14}C. Gaseous exchange was monitored during this period and the background radioactivity passing through, and impinging on, the ion chamber was determined.

The labelled bacterial preparations, as a suspension in 0·9% (w/v) NaCl, were injected into the abomasum or rumen of the sheep. The experiments are summarized in Table 1. Blood samples were withdrawn through in-dwelling jugular catheters at frequent intervals, for 24 h. Plasma was separated by centrifugation and was analysed

for total radioactivity and radioactivity in glucose. Urine and faeces were collected at 6–12 h intervals for a period of between 3 and 5 days and the radioactive content of the samples was determined.

Following injection of the bacterial preparations into the rumen, filtered rumen samples were withdrawn through a permanent rumen probe consisting of a perspex cylinder with large holes drilled in it and covered with fine nylon mesh and attached to a plastic catheter plug ($\frac{1}{8}$ in. inner diameter bore) inserted into the rumen cannula. The probe sank to the bottom of the rumen and rumen samples were obtained by a syringe attached to the end of the catheter. Plant particles were removed by centrifugation and the clear rumen liquor was assayed for total radioactivity and for radioactivity in volatile fatty acids (VFA).

Chemical methods

Total radioactivity of plasma, rumen fluid and urine was obtained by counting 0·5 ml samples in titron X-100 scintillation solution (Patterson & Greene, 1965). As at least some of the radioactivity would be expected to pass through in the faeces, samples of faeces were counted after hydrolysis in alkali as previously described. Appropriate controls were carried out to enable corrections to be applied for radioactivity adhering to fibres and for quenching due to alkali.

The concentration of glucose in plasma was determined by the method of Huggett & Nixon (1957), and glucose was isolated and assayed for radioactivity as the penta-acetate derivative (Jones, 1965). Techniques used for the determination of the specific radioactivity of rumen VFA and molar proportions of VFA have been described by Leng & Leonard (1965).

RESULTS

Chemical composition of bacterial samples

The chemical composition of the material injected into sheep is summarized in Table 2, and the detailed amino acid analysis of the radioactive samples is given in Table 3.

The amino acid content of *B. subtilis* cell walls and peptidoglycan was considerably lower than that of the other samples injected and consisted mainly of glutamic acid, alanine and diaminopimelic acid, amino acids which along with muramic acid and glucosamine made up the peptidoglycan portion of the bacterial cell. The peptidoglycan was obtained in a relatively pure form from *B. subtilis* cell walls as there were only trace amounts of other amino acids present. Some polysaccharide was still present as there was 7·5% glucose in the fraction.

Labelled samples injected into sheep alimentary tract

Mean values and standard errors for energy expenditure, respiratory quotient, plasma glucose concentration, total rumen VFA and molar proportions of acetic, propionic and butyric acid after injection of the labelled samples into the abomasum and rumen are given in Table 4.

Specific radioactivity of expired CO_2 and plasma glucose

The specific radioactivity of CO_2 in expired air, and glucose in plasma measured over a 24 h period after the injection of *B. subtilis* and *E. coli* into the rumen is shown in Fig. 1. Metabolism of carbon atoms to CO_2 in expired air was greater from *B. subtilis* than from *E. coli*, although the total contribution to the specific radioactivity of glucose was similar for both organisms.

Table 2. *Chemical composition of injection samples expressed as a percentage of the total dry weight of the sample*

Sample	Carbon content	Amino acid content	Carbohydrate content		
			Total*	Glucose	Amino sugar
Bacillus subtilis:					
Whole cells	41·3	45·6	12·1	5·4	5·3
Cell contents	43·7	43·5	9·3	2·8	1·4
Cell walls	37·4	28·0	18·6	9·8	18·2
Peptidoglycan	38·0	25·8	15·0	7·5	33·0
Escherichia coli:					
Whole cells	46·5	44·8	11·6	0·4	4·0
Cell contents	44·9	41·9	10·2	Trace	2·0
Cell walls	44·4	47·9	10·3	Trace	4·2

* Cell contents from both organisms contained approx. 1 % ribose; *E. coli* whole cells and cell walls contained approx. 1 % arabinose.

The results obtained when *B. subtilis* whole cells, cell contents and cell walls were injected directly into the abomasum are shown in Fig. 2. Radioactivity from *B. subtilis* cell contents and cell walls was incorporated into CO_2 in expired air to a lesser extent than that from whole cells. The incorporation of radioactivity into CO_2 from cell walls was slower than from cell contents. This difference in rate of incorporation was possibly reflected in the specific radioactivity of CO_2 from whole cells since there was a sustained plateau in the specific radioactivity of expired CO_2 from 2·5 to 6·5 h. Little of the ^{14}C from the cell walls was incorporated into plasma glucose.

Specific radioactivities of CO_2 in expired air and glucose in plasma after injection of *E. coli* samples into the abomasum are shown in Fig. 3. In contrast to the result obtained for *B. subtilis* cell walls, ^{14}C from *E. coli* cell walls was as extensively incorporated into glucose as ^{14}C from cell contents. There was also little difference in the extent, or rate, of conversion of ^{14}C substrate into CO_2 between cell walls and cell contents from *E. coli*. The similarity in metabolism of cell walls and cell contents from *E. coli* is in keeping with the similar chemical composition of these cell components (Table 2).

Specific radioactivity of VFA in the rumen

Fig. 4. shows the specific radioactivity of the total rumen VFA measured over a 24 h period after the injection of labelled *B. subtilis* and *E. coli* into the rumen.

Table 3. *Amino acid composition of radioactive samples*

(The amino acid content is given as a percentage of the total number of moles estimated (moles %) and as a percentage of the total dry weight of the sample (mg %). Tryptophan was destroyed during the hydrolytic procedure and only a trace of cyst(e)ine survived.)

	Bacillus subtilis								*Escherichia coli*					
	Whole cells		Cell contents		Cell walls		Peptidoglycan		Whole cells		Cell contents		Cell walls	
Amino acid	moles %	mg %	moles %	mg %	moles %	mg %	moles %	mg %	moles %	mg %	moles %	mg %	moles %	mg %
Lys	7·2	3·9	8·2	4·2	3·6	1·1	Trace		6·6	3·5	6·6	3·4	7·8	4·5
Hist	1·8	1·0	1·9	1·0	1·2	0·4	—		1·8	1·0	1·8	1·0	1·8	1·1
Arg	3·6	2·3	3·9	2·5	1·9	0·8	Trace		6·2	4·0	5·1	3·2	5·1	3·6
Asp	8·7	4·2	10·0	4·6	4·3	1·2	Trace		12·2	5·8	10·8	5·0	11·5	5·9
Thre	4·6	1·9	5·3	2·2	2·2	0·6	Trace		5·2	2·2	5·3	2·2	5·5	2·4
Ser	4·4	1·6	5·0	1·8	2·3	0·6	Trace		4·8	1·7	4·8	1·7	5·1	2·0
Glu	16·7	8·3	17·5	8·4	21·2	6·3	29·5	7·8	12·8	6·2	12·7	6·0	12·5	6·6
Pro	2·2	0·9	0·5	0·2	Trace		—		0·4	0·2	0·3	0·1	0·4	0·2
Gly	9·4	2·2	9·0	2·1	3·9	0·6	Trace		9·9	2·3	10·0	2·3	9·8	2·5
Ala	12·4	3·7	9·5	2·7	28·3	5·0	39·5	6·2	10·7	3·1	10·3	2·9	11·1	3·5
Val	6·0	2·5	7·0	2·8	2·7	0·7	—		6·5	2·7	7·0	2·8	7·1	3·1
Met + dap	4·9	3·5	3·2	1·7	19·4	8·2	31·1	11·8	2·5	1·8	2·8	1·5	2·7	2·0
Ileu	5·3	2·5	6·0	2·7	2·6	0·7	—		5·3	2·4	5·2	2·4	5·1	2·5
Leu	7·3	3·4	8·4	3·8	3·5	1·0	Trace		9·0	4·2	9·3	4·2	8·7	3·3
Tyr	2·3	1·5	1·2	0·8	1·1	0·5	—		2·7	1·8	2·2	1·4	2·6	1·9
Phen	3·2	2·3	3·5	2·0	1·6	0·6	—		3·3	2·0	3·2	1·9	3·2	2·1
Total	100	45·6	100	43·5	100	28·0	100	25·8	100	44·8	100	41·9	100	47·9

Table 4. *Mean values and standard errors for energy expenditure (cal/min), respiratory quotient, plasma glucose concentration (mg/100 ml), concentrations of volatile fatty acids (VFA) (m-moles/l.) and molar proportions (%) of VFA in the rumen of sheep*

Radioactive sample	Site of injection	Energy expenditure*	Respiratory quotient	Plasma glucose	Rumen VFA	Individual VFA					
						Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Isovaleric acid	Valeric acid
Bacillus subtilis:											
Whole cells	Abomasum	1292 ± 17	0·91 ± 0·02	75·8 ± 2·8	—	—	—	—	—	—	—
Cell contents	Abomasum	1198 ± 23	0·82 ± 0·01	66·9 ± 1·4	—	—	—	—	—	—	—
Cell walls	Abomasum	955 ± 44	0·83 ± 0·01	61·4 ± 0·8	—	—	—	—	—	—	—
Escherichia coli:											
Whole cells	Abomasum	1556 ± 62	0·81 ± 0·01	63·8 ± 5·5	—	—	—	—	—	—	—
Cell contents	Abomasum	1141 ± 30	0·81 ± 0·01	69·5 ± 4·0	—	—	—	—	—	—	—
Cell walls	Abomasum	1124 ± 31	0·81 ± 0·01	57·4 ± 2·3	—	—	—	—	—	—	—
B. subtilis:											
Whole cells	Rumen	755 ± 45	0·82 ± 0·01	60·5 ± 2·6	90·1 ± 4·6	56·0	19·3	12·8	6·7	1·9	3·3
E. coli:											
Whole cells	Rumen	815 ± 20	0·89 ± 0·01	68·1 ± 1·4	97·8 ± 2·8	66·8	18·2	9·8	1·7	1·5	1·8

* Energy expenditure was calculated from the equation given by Marston (1948) for conditions where urinary nitrogen excretion is not known.

A striking feature of the experiment was the rapidity with which ^{14}C was incorporated into VFA, the peak activity occurring at less than 2 h after the injection of the labelled cells. The incorporation from *E. coli* was not as great as that from *B. subtilis*.

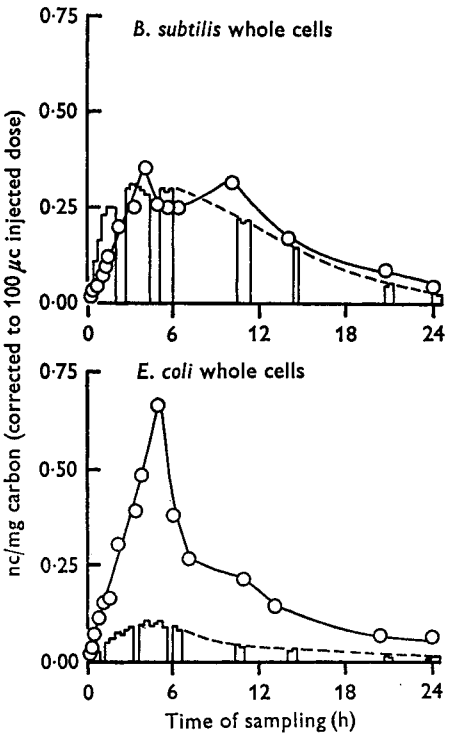

Fig. 1

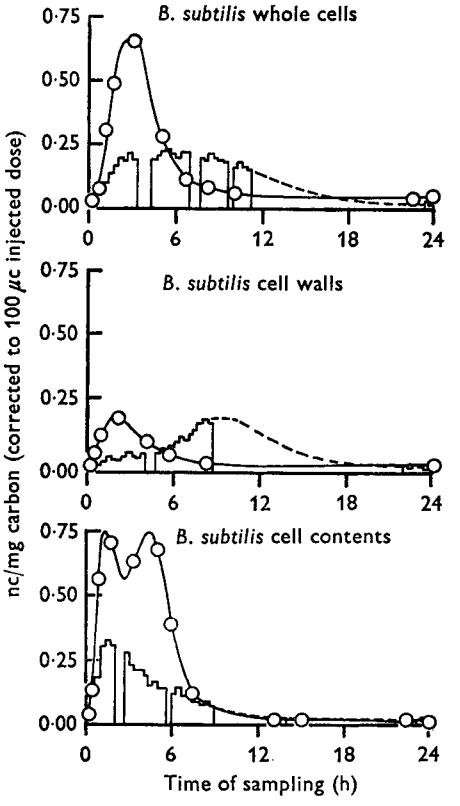

Fig. 2

Fig. 1. Specific radioactivity of expired CO_2 and plasma glucose after injection of *Bacillus subtilis* whole cells and *Escherichia coli* whole cells into the rumen of sheep. The histogram represents the specific radioactivity of CO_2 in expired air; the continuous curve the specific radioactivity of plasma glucose.

Fig. 2. Specific radioactivity of expired CO_2 and plasma glucose after injection of *Bacillus subtilis* whole cells, cell walls and cell contents into the abomasum of sheep. The histogram represents the specific radioactivity of CO_2 in expired air; the continuous curve the specific radioactivity of plasma glucose.

Recovery of radioactivity in expired air, urine and faeces

Expired air. The cumulative recovery of ^{14}C in expired air over a 24 h period after the injection of *B. subtilis* and *E. coli* into the rumen is shown in Fig. 5, and after the injection into the abomasum is shown in Fig. 6. When injected into the abomasum 16·5 and 17·5% of the ^{14}C present in *B. subtilis* and *E. coli* respectively was recovered in expired air and the rate of expiration of ^{14}C after 24 h was small. When injected into the rumen the rate of expiration of ^{14}C was slower than that observed when samples were injected into the abomasum, and it was apparent that *B. subtilis* was more extensively

oxidized than *E. coli*. Of the other samples injected into the abomasum, both *E. coli* cell walls and cell contents were oxidized to CO_2 to the same extent and *B. subtilis* cell walls were only slightly less oxidized than cell contents. The low recovery of ^{14}C in expired air from *B. subtilis* cell walls and cell contents is difficult to understand in view of the greater recovery of $^{14}CO_2$ when whole cells were injected into both the rumen and the abomasum.

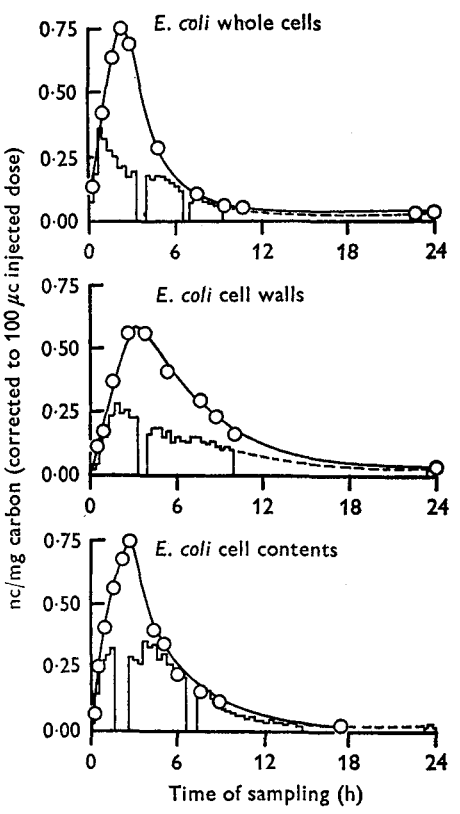

Fig. 3

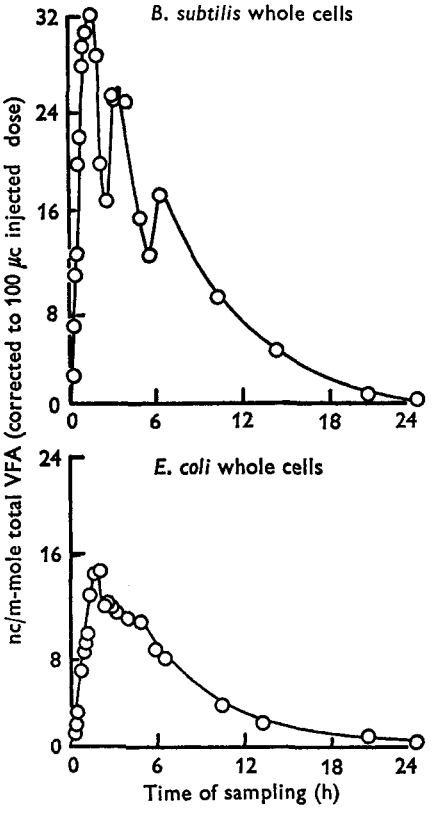

Fig. 4

Fig. 3. Specific radioactivity of expired CO_2 and plasma glucose after injection of *Escherichia coli* whole cells, cell walls and cell contents into the abomasum of sheep. The histogram represents the specific radioactivity of CO_2 in expired air; the continuous curve the specific radioactivity of plasma glucose.

Fig. 4. Specific radioactivity of total rumen volatile fatty acids (VFA) after injection of *Bacillus subtilis* whole cells and *Escherichia coli* whole cells into the rumen of sheep.

Of particular interest is the result obtained when peptidoglycan was injected into the abomasum (Fig. 6). This cell-wall component which was resistant to pepsin and trypsin (the preparative procedure included exposure to digestion by these enzymes) appears on a rate basis to be as readily oxidized to CO_2 as the cell walls from which it was prepared.

Urine and faeces. Fig. 7 shows the cumulative recovery of ^{14}C in urine and faeces after the injection of *B. subtilis* and *E. coli* whole cells into the rumen. It is obvious

that the rate of excretion of absorbed ^{14}C in urine was reaching a minimum after 50 h, but the rate of excretion of the residues in the faeces was still high. The cumulative recovery of ^{14}C in urine and faeces after injections of labelled samples into the abomasum is shown in Fig. 8. It appears that the excretion of ^{14}C was more rapid when samples were introduced into the abomasum than when they were introduced into the rumen, but that irrespective of the site, only a small proportion of the injected

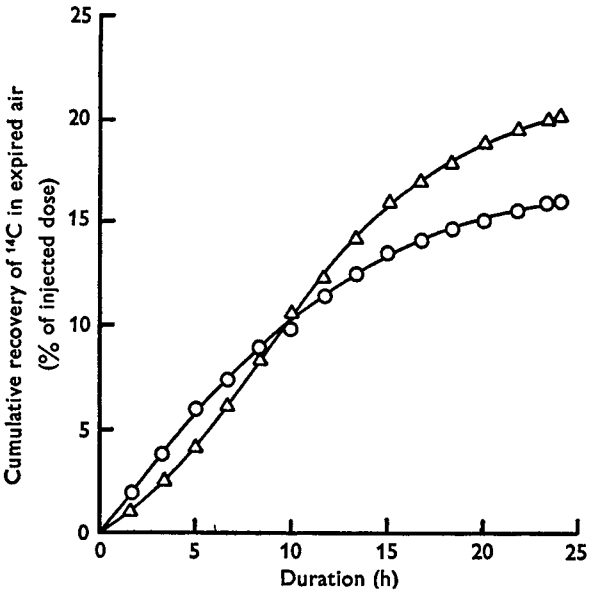

Fig. 5

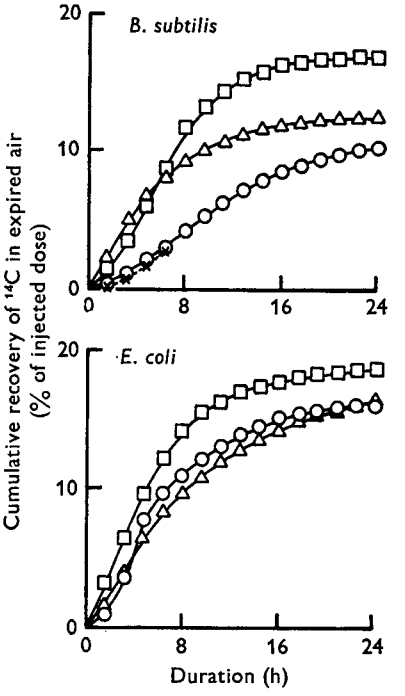

Fig. 6

Fig. 5. Cumulative recovery of ^{14}C in expired air after the injection of *Bacillus subtilis* whole cells (△—△) and *Escherichia coli* whole cells (○—○) into the rumen of sheep.

Fig. 6. Cumulative recovery of ^{14}C in expired air after the injection of *Bacillus subtilis* whole cells (□—□), cell walls (○—○), cell contents (△—△) and peptidoglycan (×—×) and *Escherichia coli* whole cells (□—□), cell walls (○—○) and cell contents (△—△) into the abomasum of sheep.

dose was recovered in urine and faeces, ranging from 17% for *E. coli* whole cells (2% in urine; 15% in faeces) to 4% for *B. subtilis* cell walls (1·5% in urine; 2·5% in faeces). The low recovery of radioactivity in faeces indicated that the true digestibility of bacterial cells is high.

DISCUSSION

Experiments have been done to determine the nutritive value of rumen bacteria (Johnson, Hamilton, Robinson & Garey, 1944; Reed, Moir & Underwood, 1949; McNaught, Smith, Henry & Kon, 1950) by feeding bacterial preparations to rats. The results indicated that rumen bacteria are of high nutritive and biological value

to rats and it was inferred that they are of similar value to sheep. The chemical composition of bacteria indicates that they are potential sources of many nutrients. Thus, bacteria and cell walls from rumen bacteria were rich in protein and lipid, which

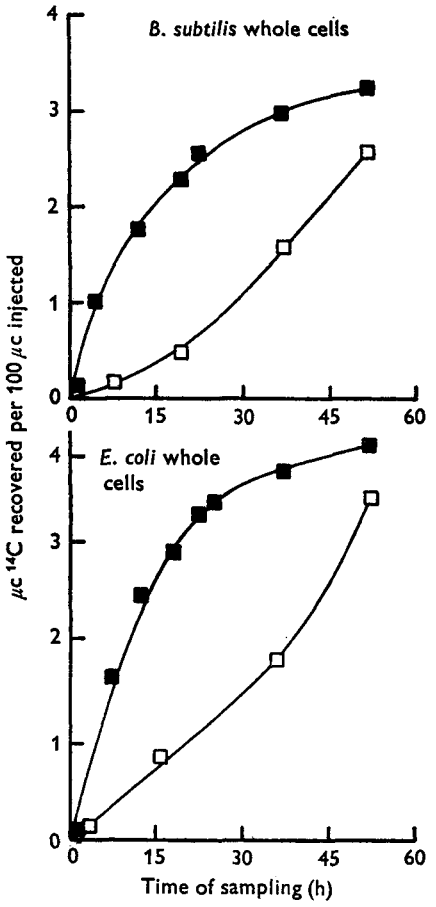

Fig. 7

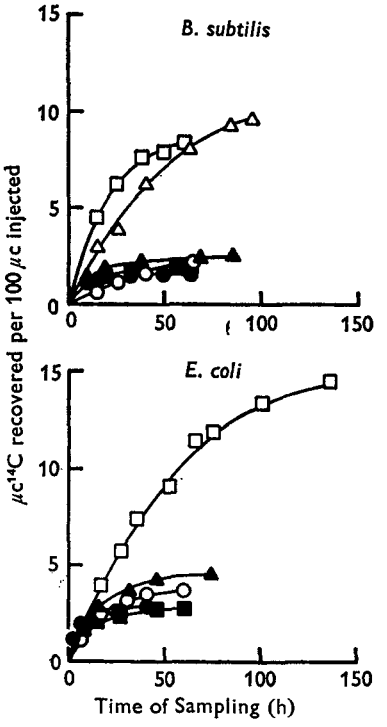

Fig. 8

Fig. 7. Percentage recovery of ^{14}C in urine (■—■) and faeces (□—□) after the injection of *Bacillus subtilis* whole cells and *Escherichia coli* whole cells into the rumen of sheep.

Fig. 8. Percentage recovery of ^{14}C in urine and faeces after the injection of *Bacillus subtilis* whole cells, cell walls and cell contents and *Escherichia coli* whole cells, cell walls and cell contents into the abomasum of sheep. ■—■, whole cells, urine; □—□, whole cells, faeces; ●—●, cell walls, urine; ○—○, cell walls, faeces; ▲—▲, cell contents, urine; △—△, cell contents, faeces.

together accounted for 50–70% of the dry weight of the samples (Hoogenraad & Hird, 1970). It has also been determined that bacterial autolytic enzymes, digestive enzymes and lysis by bacteriophages break down the various bacterial polymers (N. J. Hoogenraad & F. J. R. Hird, unpublished results to be submitted elsewhere). Protozoa have also been shown to digest a wide variety of bacteria including *E. coli* (Coleman, 1967). The present paper deals with the actual utilization of bacteria in vivo.

Whereas it would be desirable to use rumen bacteria in such a study, practical and nutritional problems make it difficult to culture labelled bacteria which would be representative of the microflora of the rumen. As large quantities of bacteria of high specific radioactivity were required it was decided that *E. coli* and *B. subtilis*, being representative of Gram-negative and Gram-positive organisms, would be suitable for these studies. A chemical analysis of the labelled samples used showed that both *B. subtilis* and *E. coli* whole cells were similar to rumen bacteria. The cell walls prepared from *E. coli* also were similar to cell walls prepared from rumen bacteria, although the amino acid content of *E. coli* cell walls was 10% greater than that from rumen bacteria.

Table 5. *Percentage of radioactivity, injected into sheep, recovered in expired CO_2, urine and faeces, ^{14}C not excreted and the digestibility of bacterial samples*

Experiment	Percentage recovery of ^{14}C*			^{14}C not excreted or metabolized to CO_2	Digestibility† (%)
	Expired CO_2	Urine	Faeces		
Bacillus subtillis:					
Whole cells into the rumen	20·5	3·5	6·0‡	70·0	94·0
Escherichia coli:					
Whole cells into the rumen	16·5	5·0	8·0‡	70·5	92·0
B. subtilis:					
Whole cells into abomasum	16·5	2·0	9·0	72·5	91·0
Cell walls into abomasum	10·5	1·5	2·5	85·5	97·5
Cell contents into abomasum	12·0	2·5	11·0	74·5	89·0
E. coli:					
Whole cells into abomasum	17·5	2·0	15·0	65·5	85·0
Cell walls into abomasum	16·5	3·0	4·0	77·5	96·0
Cell contents into abomasum	16·5	4·5	ND	—	—

ND. Not determined.
* Obtained by extrapolation of curves obtained in Figs. 5–8.
† Calculated from the amount of ^{14}C excreted in faeces.
‡ Incomplete excretion: see Fig. 7.

Digestion of bacterial samples in the abomasum and the alimentary tract distal to the abomasum was shown to occur by the incorporation of ^{14}C into expired CO_2 and plasma glucose when samples were injected into the abomasum. When injected into the rumen, label from *B. subtilis* and *E. coli* was rapidly incorporated into VFA showing that the bacterial samples were degraded in this organ also. Portugal & Sutherland (1966) found that label from [^{14}C]glutamic acid and [^{14}C]aspartic acid was rapidly incorporated into CO_2 and VFA when injected into the rumen of sheep, and it is likely therefore that some of the label in VFA is due to metabolism of amino acids of bacterial origin in the rumen.

The digestion of peptidoglycan when injected into the abomasum raises the question of the presence of bacteriolytic enzymes in sheep intestine, as this cell-wall polymer is resistant to digestion by trypsin (Salton & Pavlik, 1960).

Injection of cell walls from *B. subtilis* and *E. coli* into the abomasum showed that this portion of the bacterial cell was also readily digested and metabolized. Thus, in

Table 6. *Contribution of bacterial carbon to the glucose synthesis in sheep*

Experiment	Mean specific radioactivity of plasma glucose (μc/g C)* (*A*)	Mean radio-activity in glucose pool† (μc) (*B*)	Mean rate of flow of ^{14}C through glucose pool‡ (nc/min) (*C*)	Percentage of bacterial ^{14}C flowing through glucose pool in 24 h	^{14}C in bacterial components oxidized via glucose§ (% injected dose) (*D*)	Percentage of expired ^{14}C arising from glucose oxidation‖
Bacillus subtilis:						
Whole cells into abomasum	0·122	0·223	3·1	4·6	1·4	8·5
Cell contents into abomasum	0·183	0·312	4·4	6·3	2·0	16·7
Cell walls into abomasum	0·069	0·124	1·7	2·4	0·7	6·7
Escherichia coli:						
Whole cells into abomasum	0·121	0·218	3·0	4·3	1·3	7·4
Cell contents into abomasum	0·157	0·283	3·9	5·6	1·9	11·5
Cell walls into abomasum	0·220	0·396	5·6	8·1	2·6	15·7
B. subtilis:						
Whole cells into rumen	0·210	0·380	5·3	7·7	2·5	12·2
E. coli:						
Whole cells into rumen	0·185	0·333	4·7	6·6	2·1	12·7

* Obtained by integration of the glucose specific radioactivity *v.* time curves by cutting and weighing (Figs. 1–3).

† *A* multiplied by the mean pool size of 4·52 g glucose or 1·8 g glucose C (White, Steel, Leng & Luick, 1969).

‡ Mean irreversible loss of glucose, 63·7 mg/min (White *et al.* 1969) was expressed as a percentage of the pool size per min, namely 63·7/4520 = 1·4 %. Therefore $C = B \times 1{\cdot}4/100$.

§ Oxidation rate of glucose was taken as 32 % of irreversible loss (Bergman, 1963; Bergman, Roe & Kon, 1966; Annison, Brown, Leng, Lindsay & West, 1967). Therefore $D = C \times 32/100$.

‖ *D* expressed as a percentage of the cumulative recovery of ^{14}C via expired air over 24 h (see Figs. 6 and 7).

seven experiments no more than 15% of the injected dose was recovered in the faeces (Table 5), emphasizing the high digestibility in the lower gut of all the bacterial preparations.

From published experimental data (Table 6) it was possible to estimate the amount of bacterial carbon flowing through the glucose pool of the sheep in each of the experiments. It was shown that a portion of the ^{14}C of bacterial samples was incorporated into glucose. Where the labelled material was injected into the abomasum some glucose may have originated directly from bacterial polysaccharide. However, some differences were apparent. *B. subtilis* cell walls contained 10% glucose, but only 2·4% of the bacterial carbon flowed through the glucose pool in 24 h. In contrast, *E. coli* whole cells, cell walls and cell contents all contained less than 1% glucose, yet up to 8% of the bacterial carbon (from *E. coli* cell walls) flowed through the glucose pool and therefore most of the label in plasma glucose must have arisen by gluconeogenesis.

A large proportion of bacterial matter was oxidized by pathways other than through glucose. When expressed as a percentage of the cumulative recovery of ^{14}C via expired CO_2 it can be seen that only 7·7–17·2% of the bacterial components were oxidized via glucose.

It is apparent also that a large proportion of the injected dose was not recovered in expired air, urine or faeces over the duration of the experiment (Table 5). It appears likely that this was due to an incorporation of the ^{14}C into body constituents, such as proteins and lipids and a retention of ^{14}C compounds in body fluid pools.

An estimate of the contribution which rumen bacteria may make to the glucose requirements of sheep can be made. Thus, if the carbon content of the bacteria was 44% (average C content of *E. coli* and *B. subtilis* whole cells, Table 2) and if 5·8% of the bacterial carbon passed through the glucose pool in 24 h (average result obtained when *E. coli* and *B. subtilis* whole cells were injected into the rumen and abomasum, Table 6), then 100 g dry weight of bacteria would give rise to 2·6 g carbon flowing through the glucose pool in 24 h, which is equivalent to 6·6 g glucose. This estimate, however, represents the maximum likely contribution as it includes the incorporation of ^{14}C from precursors which do not result in a net synthesis of glucose.

Hungate (1966) calculated that sheep would receive 100 g protein/day from bacterial sources, and this is in fair agreement with the results of Walker & Nader (1968). Taking the average amino acid content of rumen bacteria as 38% of the dry matter (Hoogenraad & Hird, 1970) this amount of microbial protein would be equivalent to about 260 g bacterial dry matter. From our present experiments this in turn would be expected to give rise to 17 g glucose. Since sheep under similar conditions have been shown to have a total glucose entry rate of 92 g/day (White *et al.* 1969), it appears that about 18·5% of this may come from bacteria.

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