

Utilization of ^{14}C -labelled *Escherichia coli* by rats

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1. *Escherichia coli* was grown on ^{14}C -labelled glucose and fractions representing 'whole cells', 'cell contents' and 'cell walls' were administered orally to rats.
2. ^{14}C appearing as $^{14}\text{CO}_2$ in the expired air and as unidentified radioactive products in urine and faeces was measured until the cumulative recovery showed little change with time.
3. All fractions were digested. The digestibility of cell walls was less than that of the other fractions.
4. There was considerable variation among individual rats.

It is common knowledge that ruminants are able to derive benefit from the synthetic potential of the microbial content of the rumen. Preparations from ^{14}C -labelled *Bacillus subtilis* and *Escherichia coli* of 'whole cells', 'cell contents' and 'cell walls' that are almost completely digested by the sheep have been described (Hoogenraad, Hird, White & Leng, 1970).

Some of the methods suggested for relieving the world shortage of protein for human nutrition depend on its production by various fermentation processes. Considerable expenditure is required for these, and it is important to develop reliable and quick methods for measuring digestibility so that the nutritional value of the product can be related to the cost of the procedures used.

The rat has the ability to digest whole cells of *E. coli*; this has been studied (Kaufman, Nelson, Brown & Forbes, 1957) using the classical methods of Mitchell (1924). These methods are time-consuming and the results we report may contribute to the design of simpler procedures that are suitable for routine testing of microbial products.

EXPERIMENTAL

Preparation of radioactive bacterial samples

E. coli was cultured on the mineral salts medium C of Roberts, Abelson, Cowie, Bolton & Britten (1955) with glucose (2 g/l) as the sole C source, and 0.05 mCi [U- ^{14}C]glucose/l culture medium, as described by Coleman (1967). Cells were grown in an aerated fermenter at 38° for 24 h in a 1 l batch, harvested and washed in water. They were treated as described by Hoogenraad *et al.* (1970). A portion was immediately freeze-dried as 'whole cells'. The remaining cells were disrupted using a French Press (pressure cell (American Instrument Co. Inc., Silver Spring, Maryland,

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USA) in an hydraulic press described by Vanderheiden, Fairchild & Jago (1970) and 'cell contents' were separated from 'cell walls' by centrifugation at 20000 g for 30 min. After washing the 'cell wall' pellet with saline solution (9 g NaCl/l) and water, both fractions were freeze-dried.

Six male rats of the Buffalo strain (Abbots Farm, Eltham, Victoria, Australia), about 3 months of age and weighing about 250 g, were used. They were housed singly in an all-glass metabolism cage (Jencons Metabowl; Jencons Scientific Ltd, Hemel Hempstead, Herts., UK) and given free access to water and to the colony diet. This consisted of a commercial dog ration containing (g/kg): 100 moisture, 600 carbohydrate, 200 crude protein (nitrogen $\times 6.25$), 50 crude fibre, 40 crude fat, 10 salt; fortified with vitamins (Fido Dog Cubes; Barastoc Products, Echuca, Victoria, Australia). The temperature varied between 20 and 28° during the experiments.

The animals were each made accustomed to their metabolism cage for a few days before the experiment began. Samples of the preparations in 35 mg portions (equivalent to 0.37 $\mu\text{Ci }^{14}\text{C}$) were mixed with the minimum quantity of honey and given orally to the rats. Urine and faeces were collected daily for 5 d. Expired carbon dioxide was collected on the 1st day, from the 2nd to the 5th day and from the 6th to the 12th day. The three preparations were given to each of the six rats in a different order. At least 5 d elapsed between experiments on the same animal. Only one metabolism cage was available and although the rats were matched initially according to age and weight, it was not possible to replicate the dietary treatments on each rat due to weight increases.

Radioactivity measurements

Expired CO_2 was collected in 2 M-NaOH solution and 0.5 ml samples were added to an ethanol-toluene scintillation solution containing 75 g Cab-O-Sil (Godfrey L. Cabot, Boston, Massachusetts, USA)/l, as described by Harlan (1961).

The nature of the radioactive products measured in urine and faeces was not studied. The daily collection of urine was diluted to constant volume with water and 0.5 ml samples were counted in a dioxane scintillation solution (Bray, 1960) containing 50 g Cab-O-Sil/l. The samples of faeces were dried to constant weight, hydrolysed in alkali as described by Hoogenraad *et al.* (1970) and 0.5 ml samples were counted in the same medium as used for urine. Some microphosphorescence was noted in the vials (cf. De Ment, 1945). Reproducible results were obtained after the vials had been left for 1 or more d.

RESULTS

Food intake for the experimental period varied from 5 to 11 g/d, averaging 8 g/d, but there was no evidence that the variation was associated with the different bacterial preparations given.

The cumulative recoveries of radioactivity as $^{14}\text{CO}_2$ in expired air, faeces and urine, with time after administration of the dose, are summarized in Fig. 1. It can be seen that the division of the ingested material between excretion and incorporation into storage pathways was 90% complete within 2 d.

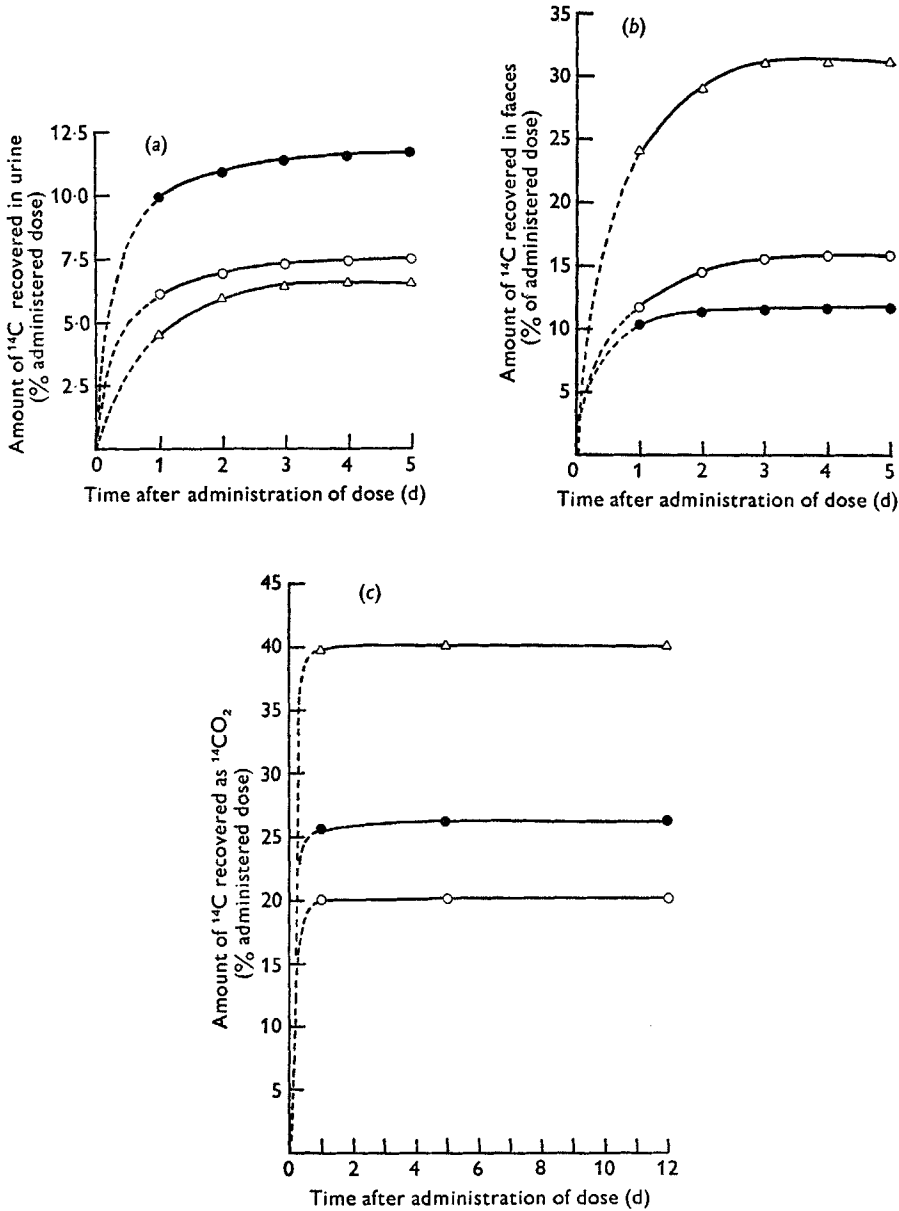


Fig. 1. Cumulative recovery of ¹⁴C in (a) urine, (b) faeces and (c) as ¹⁴CO₂ in expired air of rats after oral administration of preparations of 'whole cells' (○), 'cell contents' (●) and 'cell walls' (△) from *Escherichia coli* grown on ¹⁴C-labelled glucose; for details of preparation of cell fractions, see p. 175. Each point represents the mean value for six animals for urine and faeces; for ¹⁴CO₂ in expired air, the points represent values for individual rats each given one preparation.

Table 1. *Recovery of ^{14}C in urine and faeces and as $^{14}\text{CO}_2$ in expired air of rats after oral administration of preparations of 'whole cells', 'cell contents' and 'cell walls' from Escherichia coli grown on ^{14}C -labelled glucose, and digestibilities for the three fractions*

Fraction* administered	Amount of dose recovered (% administered dose) in:			Amount of dose retained			Digestibility ratio†
	Urine	Faeces	$^{14}\text{CO}_2$	% Administered			
				dose	Angle mean†	SE	
'Whole cells'	7.5	15.5	28.5	48.5	44.04	2.58	0.845
'Cell contents'	11.5	13.1	40.1	35.3	36.18	3.40	0.869
'Cell walls'	6.5	31.0	34.0	28.5	31.44	2.92	0.690

* For details of preparation, see p. 175.

† Transformed values for the percentage amount of ^{14}C assimilated; for details, see Snedecor (1946).

‡ Calculated from the amount of ^{14}C excreted in the faeces.

The average recoveries of radioactivity for the three preparations for the experimental period are given in Table 1.

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The amount of ^{14}C recovered in faeces was greater for 'cell walls' than for 'whole cells' or 'cell contents'. Taking into account the weight of 'cell walls' (about 160 mg/g dry weight) and the values for digestibility obtained for 'cell walls' and 'cell contents', one would predict a value for digestibility for the 'whole cells' of 0.84; a value almost identical with that found.

Further information was obtained from an analysis of variance of the amount of ^{14}C which was assimilated into pathways from which it was not released within the experimental period, i.e. it was not recovered in urine, faeces or expired air. The values for the percentage amount of ^{14}C assimilated were transformed to angle means by the method of Snedecor (1946). The differences between animals and between preparations were both statistically significant ($P < 0.01$). The residual variance would have included physiological factors not mentioned above; any effects of temperature variations during the experimental period and small manipulative and analytical errors.

Comparisons of the transformed data by the t test showed that assimilation from 'whole cells' was significantly greater than that from 'cell contents' ($P < 0.5$) and from 'cell walls' ($P < 0.01$). Assimilation from 'cell contents' did not differ from that found for 'cell walls'.

DISCUSSION

Our results for digestibility for the 'whole cell' preparations from *E. coli* do not differ from those found by the older method used by Kaufman *et al.* (1957) although the variance is such that small differences would not be indicated.

Bacterial cell walls contain a number of bonds for which the non-ruminant mammal has no specific lytic enzyme. In ruminants, Hoogenraad *et al.* (1970) consider that bacterial autolytic enzymes, proteolytic enzymes and lysis by bacteriophages, act in

concert to break down the various polymers in the cell wall. Coleman (1967) has reported that protozoa can digest a wide variety of bacteria, including cells of the species used here. De Petris (1967) has found that the membrane and cell wall components of *E. coli* can be partly digested in vitro through the combined efforts of lysozyme and proteolytic enzymes of non-ruminants. Deckx, Vantrappen & Parein (1967) have found that Paneth cell granules isolated from the small intestine of rats have considerable lysosomal activity.

The fact that 'whole cells' were better assimilated than 'cell contents' or 'cell walls' was unexpected. It would be consistent with the view that some autolytic digestion of 'cell walls' by 'cell contents' may take place in the gut when 'whole cells' are fed. However, our findings could equally be due to differences between animals, which were found to be significant by analysis of variance but which are included in the *t* test.

It was originally hoped to continue the work by studying the effects of restricting food intake on the metabolism of the microbial products. However, it was not found possible to monitor the intake when the animals were given access to food within the cage without opening the apparatus.

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