The Metabolism of Starch, Glucose, Amino Acids, Purines, Pyrimidines and Bacteria by the Rumen Ciliate *Polyplastron multivesiculatum*

By G. S. COLEMAN AND JUDITH I. LAURIE

Agricultural Research Council, Institute of Animal Physiology,
Babraham, Cambridge CB2 4AT

(Received 19 May 1976)

SUMMARY

The large rumen ciliate protozoon *Polyplastron multivesiculatum* grown *in vitro* engulfed a wide range of bacteria (from a population density of 10^9 bacteria ml^{-1}) at a rate of 1500 to 137000 bacteria h^{-1} protozoon $^{-1}$. No evidence was found for the preferential engulfment of bacteria of rumen origin. Except for *Proteus mirabilis*, none of the bacteria were digested with the liberation of soluble materials into the medium. Glucose and amino acids were taken up rapidly by *P. multivesiculatum* compared with the rate of uptake by *Entodinium caudatum*. Glucose was incorporated into protozoal polysaccharide and into bacteria associated with the protozoa and was used for the synthesis of a wide range of amino acids. Evidence showed that bacteria and free amino acids at the concentrations found in the rumen could supply the protein requirements of the protozoa for division at least once each day.

INTRODUCTION

The rumen Entodiniomorphid protozoa *Entodinium caudatum* and *Ento. simplex* rapidly engulf particulate matter (Coleman & Hall, 1969). They utilize starch grains as a source of glucose for energy, and bacteria as a source of amino acids and nucleotides for protein and nucleic acid synthesis (Coleman, 1964, 1967*b*, 1968, 1969, 1972). In contrast, *Epidinium ecaudatum caudatum* takes up bacteria comparatively slowly and utilizes free amino acids and plant protein as sources of amino acids for growth (Coleman & Laurie, 1974). This paper describes the metabolism of the large rumen protozoon *Polyplastron multivesiculatum* which, when grown on a potassium phosphate-rich medium, has an obligate requirement for certain other protozoa e.g. *Epidinium* spp., but grows on sodium chloride-rich medium in the absence of other protozoa (Coleman, Davies & Cash, 1972).

METHODS

Organism. *Polyplastron multivesiculatum* was grown either in the presence of *Epi. ecaudatum caudatum* or on Hungate-type salts medium (Coleman *et al.*, 1972). Washed suspensions were prepared as described by Coleman (1969) except that mineral salt solution D (Coleman, 1972) was used throughout. This protozoon sedimented rapidly in salts medium, and so it was difficult to separate it from the plant material on which it grew: all suspensions were therefore heavily contaminated with grass and husk from the wholemeal flour on which it was fed.

Incubation conditions. The medium contained: 0.05 to 0.5 ml ^{14}C -labelled sugar, amino acid, purine or pyrimidine (0.005 to 1.0 M; 1.0 to 25 $\mu\text{Ci ml}^{-1}$) or ^{14}C -labelled bacteria;

0 to 0.04 ml 0.3 % (w/v) chloramphenicol; 1.0 ml suspension of intact, or where indicated, sonicated protozoa (300 to 2000 ml⁻¹); and 0 to 0.45 ml salt solution D; to give a final volume of 1.5 ml. Thick-walled test tubes (100 × 13 mm) were used; after inoculation the contents were gassed for 15 s with N₂/CO₂ (95:5, v/v), and the tube was sealed with a rubber bung and incubated at 39 °C. After incubation, the intact protozoa were centrifuged and washed three times in salt solution D on a swing-out-head centrifuge for 30 s from rest (maximum speed equivalent to 200 g). The washed protozoa were then either plated for the determination of radioactivity (Coleman, 1969) or suspended in 2 ml H₂O and disrupted.

Disruption of protozoa. The tube containing washed protozoa was immersed, to the depth of the liquid in the tube, in an 80 kHz 40 W ultrasonic cleaning bath (KG 80/1; Kerry's, Chester Hall Lane, Basildon, Essex) until no intact protozoa could be detected microscopically. The crude homogenate was centrifuged at 7000 g for 20 min: the supernatant fluid from this centrifugation was termed the broken-cell supernatant fluid and the pellet, after washing once, was the broken-cell pellet.

When the protozoa were sonicated before incubation, the homogenate was centrifuged and the pellet was washed three times in salt solution D at 7000 g for 20 min before plating out for determining radioactivity. This pellet, which is the same as the broken-cell pellet referred to above, contained any bacteria and polysaccharide granules present in the protozoa.

Culture of rumen bacteria. These bacteria were grown on medium 2 of Hobson (1969) but with the addition of [8-¹⁴C]guanine (0.1 µCi; 0.4 µg ml⁻¹).

All other techniques were as described by Coleman (1967*a, b*, 1968, 1969, 1972) and Coleman *et al.* (1972).

RESULTS

Engulfment of bacteria

Polyplastron multivesiculatum, grown in the presence or absence of an *Epidinium* sp., engulfed all the ¹⁴C-labelled bacteria tested at a constant rate for at least 3 h. The rate at which different bacteria were engulfed was compared on the basis of (i) the number of bacteria taken up per protozoon at an infinitely high bacterial suspension density, and (ii) the volume of medium cleared of bacteria by each protozoon from an infinitely dilute bacterial suspension as described by Coleman (1972). The maximum rate at which bacteria were engulfed varied markedly between species, with 100-fold variation between bacteria of a similar size, e.g. *Butyrivibrio fibrisolvens* and *Proteus mirabilis* (Table 1). However, the variation was much less with 10⁹ bacteria ml⁻¹ and was only sevenfold on the basis of the volume of medium cleared of bacteria. No evidence was obtained for any marked preferential uptake of those organisms of rumen origin, i.e. *Butyrivibrio fibrisolvens*, *Torulopsis glabrata* and mixed rumen bacteria. The species taken up most rapidly was *Pr. mirabilis* which is found in the protozoal culture medium. The uptake of this bacterium by *P. multivesiculatum* was, like its uptake by *Epi. ecaudatum caudatum* (Coleman & Laurie, 1974), almost proportional to the bacterial population density, whereas the volume of medium cleared of bacteria was almost independent of the number of bacteria present. In contrast, the uptake of *B. fibrisolvens* was comparatively unaffected by the bacterial population density, but the volume of medium cleared of bacteria decreased rapidly with decreasing bacterial density.

As, in addition to other cellular inclusions such as polysaccharide granules, the engulfed bacteria sometimes occupied over 50 % of the volume of the protozoon, it is likely that with *Pr. mirabilis*, at least, the number engulfed could be limited by the volume available.

Table 1. *The uptake of bacteria by Polyplastron multivesiculatum*

The maximum number of bacteria engulfed is the number taken up from an infinitely dense bacterial suspension. The maximum volume of medium cleared of bacteria is the volume cleared when the bacterial suspension was infinitely dilute.

Bacterium	Protozoal growth conditions	Bacteria engulfed h ⁻¹ protozoon ⁻¹		10 ⁻⁶ × Volume cleared of bacteria h ⁻¹ protozoon ⁻¹	
		Maximum	At 10 ⁹ bacteria ml ⁻¹	Maximum (μm ³)	At 10 ⁹ bacteria ml ⁻¹ (μm ³)
<i>Bacillus megaterium</i>	H	3 100	1 530	80	12
	E	17 000	10 500	51	7.1
<i>Bacteroides ruminicola</i>	E	57 000	9 500	14	8.5
<i>Butyrivibrio fibrisolvens</i>	H	7 050	6 550	100	6
	E	32 000	8 900	19	10
<i>Escherichia coli</i>	H	15 200	9 600	30	9
	E	230 000	47 000	50	43
<i>Klebsiella aerogenes</i>	H	27 000	5 600	12	6
<i>Proteus mirabilis</i>	H	1 080 000	58 000	40	39
	E	1 500 000	137 000	14	13
<i>Selenomonas ruminantium</i>	E	35 000	15 000	27	15
<i>Streptococcus faecalis</i>	H	780 000	66 000	150	15
<i>Torulopsis glabrata</i>	H	10 000	6 000	20	ND
	E	24 000	13 000	23	ND
Mixed rumen bacteria	H	97 000	8 900	15	10
	E	83 000	12 000	17	13

H, Protozoa grown in the absence of other protozoa; E, protozoa grown in the presence of an *Epidinium* sp.; ND, not determined.

Some bacteria were bound non-specifically to the plant material in the suspensions but all the results are corrected for the uptake obtained on incubation at 0 °C when the protozoa were inactive, and for that obtained initially when the protozoa and bacteria were mixed together and separated as soon as possible. The rates of uptake under these conditions were approximately 30 % and 20 % respectively of those found on incubation for 4 h at 39 °C.

As the volume of *P. multivesiculatum* grown in the presence or absence of an *Epidinium* sp. was 2.3×10^6 or 0.93×10^6 μm³ respectively, more bacteria should be taken up by protozoa grown under the former than the latter conditions. The volume available for the engulfment of bacteria was probably less than the difference in the volume of the protozoa grown under the two conditions because *P. multivesiculatum* grown in the presence of an *Epidinium* sp. always contained at least one of these protozoa and much storage material. However, as the increase in the number of bacteria engulfed by protozoa in the presence of an *Epidinium* sp. was greater than the increase in the volume, some other factor must also determine the number of bacteria taken up.

Digestion of bacteria. Of the bacteria tested (Table 1), only *Pr. mirabilis* was digested by the protozoa with the release of low molecular weight compounds into the medium: after incubating 100 protozoa ml⁻¹ (grown in the presence of an *Epidinium* sp.) with 3.5×10^9 ¹⁴C-labelled bacteria ml⁻¹ for 6 h, ¹⁴C equivalent to 650 000 bacteria was found in each protozoon and ¹⁴C equivalent to 2.0×10^6 bacteria/protozoon was found free in the medium. With the other bacteria, the amount of ¹⁴C found in the medium after 3 h incubation was often less than that in the absence of protozoa, suggesting that the protozoa took up ¹⁴C-labelled compounds liberated from the bacteria. Average uptakes over 3 h, measured as the

Table 2. *The uptake of free amino acids by Polyplastron multivesiculatum grown in the presence or absence of an Epidinium sp.*

Protozoa were incubated anaerobically either for 1 h in salt solution containing the uniformly ^{14}C -labelled amino acid under investigation at 0.1 mM, or for 6 h in salt solution containing chloramphenicol ($80\ \mu\text{g ml}^{-1}$) with the addition of 0.1 mM-amino acid after 5 h. The amount of amino acid taken up was calculated from the ^{14}C content of protozoa and the known specific activity of the amino acid added. The results are expressed in $\mu\text{mol h}^{-1}$ (10^6 protozoa) $^{-1}$.

Amino acid	Protozoa grown with <i>Epidinium</i> sp.		Protozoa grown without <i>Epidinium</i> sp.
	No pre-incubation	Pre-incubated with chloramphenicol	No pre-incubation
L-Ala	7.4	2.5	0.81
L-Arg	8.3	6.6	1.17
L-Asp	6.5	1.5	0.58
L-Glu	8.0	0.8	0.86
Gly	2.6	1.2	0.91
L-Ile	ND	ND	0.47
L-Lys	6.6	2.7	0.87
L-Met	ND	ND	0.48
L-Phe	ND	ND	0.52
L-Pro	ND	ND	0.52
L-Ser	2.9	1.3	1.34
L-Val	3.0	0.65	0.64

ND, not determined.

number of bacteria from which the soluble compounds were derived, were (in bacterial equivalents/protozoon) 28000 from *Escherichia coli*, 20000 from *Bacillus megaterium*, 100000 from *Streptococcus faecalis*, 50000 from *Klebsiella aerogenes*, and 200000 from mixed rumen bacteria. There was considerable variation between experiments and no consistent differences between protozoa grown under different conditions. Digestion of ^{14}C -labelled bacteria was also measured by the rate of appearance of ^{14}C in the protozoal broken-cell supernatant fluid. For all the bacteria tested (Table 1), 25 to 40 % of the protozoal ^{14}C was found in this fraction after 3 h, suggesting that some of the bacteria were being digested.

When ^{35}S -labelled rumen bacteria were disrupted ultrasonically (Coleman & Laurie, 1974) to produce an almost clear solution which was then incubated with the protozoa, the rate of uptake of ^{35}S compared with that from intact bacteria was increased by $65 \pm 53\%$ ($n = 4$). This suggested that *P. multivesiculatum* took up sub-bacterial particles more rapidly than intact bacteria.

Attempts to show the transfer of ^{14}C -labelled amino acids from bacterial to protozoal protein were only partially successful due to the small number of protozoa available, but it was demonstrated by autoradiography of two-dimensional paper chromatograms that [^{14}C]glycine and [^{14}C]alanine from *Pr. mirabilis* and *K. aerogenes* respectively were transferred unchanged into protozoal protein.

Metabolism of amino acids

All the ^{14}C -labelled amino acids tested (at 0.1 to 10 mM) were taken up by suspensions of *P. multivesiculatum* over 6 h. Unfortunately, the investigations were hampered because suspensions of protozoa grown in the presence of epidinia contained plant material which was contaminated with bacteria that took up amino acids. To resolve this problem, protozoal suspensions were incubated for 1 h in the absence, or 6 h in the presence, of $80\ \mu\text{g}$ chlor-

Fig. 1. Effect of substrate concentration on the incorporation of [U- ^{14}C]glucose into intact *Polyplastron multivesiculatum* (●) and the broken-cell supernatant fluid (○) derived therefrom by ultrasonic disruption and centrifuging at 7000 g for 20 min.

amphenicol ml^{-1} (to inhibit bacterial protein synthesis) and ^{14}C -labelled amino acid was added either initially or after 5 h (Table 2). Under these conditions, in 4 h the bacteria from 10^6 protozoa took up 5.5 or 29 μmol L-isoleucine, respectively, from 0.05 mM -L-[U- ^{14}C]-isoleucine in the presence or absence of chloramphenicol (80 $\mu\text{g ml}^{-1}$), indicating that the antibiotic inhibited uptake of this amino acid by the bacteria.

Of the ^{14}C taken up from the ^{14}C -labelled amino acids, 25 to 50 % appeared in the broken-cell supernatant fluid and, of this, 50 % was in the cold-TCA-soluble pool and 30 % in the protein fraction. In the broken-cell pellet, 60 % of the ^{14}C was in the protein fraction. Most radioactivity was in the original amino acid but some was always found in a few other metabolically related amino acids.

Metabolism of purines and pyrimidines

The protozoa incorporated ^{14}C from [8- ^{14}C]adenine, [8- ^{14}C]guanine and [2- ^{14}C]uracil. After 4 h most of the ^{14}C (68 to 76 %) was always in the broken-cell supernatant fluid and, of this, 13 to 25 % was present in the nucleic acid fraction. Adenine and guanine were metabolically interconvertible and both gave rise to xanthine and hypoxanthine in the medium which were then metabolized at the C-8 position to give presumably $^{14}\text{CO}_2$. Uracil was partially converted to cytosine in the protozoa and was metabolized at the C-2 position to give, probably, $^{14}\text{CO}_2$.

Metabolism of glucose

Polyplastron multivesiculatum took up ^{14}C linearly from 0.67 mM -[U- ^{14}C]glucose for at least 1 h at a rate of 40 $\mu\text{mol h}^{-1}$ (10^6 protozoa) $^{-1}$. In the presence of chloramphenicol (80 $\mu\text{g ml}^{-1}$) this rate decreased by 15 %, and by 53 % if the protozoa were pre-incubated with the antibiotic for 5 h. However, incorporation into the broken-cell supernatant fluid was unaffected, suggesting that the antibiotic only affected incorporation by bacteria. Of the ^{14}C recovered after uptake of 0.038 μmol [U- ^{14}C]glucose by 500 protozoa during 4 h in the absence of antibiotic, 61 % was present in the broken-cell supernatant fluid. This was distributed between nucleic acids (15 %), protein (19 %) and other soluble substances (66 %). In the broken-cell pellet obtained after incubation in the presence of chloramphenicol,

18 % of the ^{14}C was in protein and 70 % in the hot-TCA-soluble fraction. After complete acid hydrolysis of the latter fraction, over 90 % of the ^{14}C was present as glucose; 30 % of this was from the protozoal polysaccharide granules, as determined by the method of Coleman & Laurie (1974), and the remainder was probably from bacterial capsular polysaccharide, as was found with *Entodinium caudatum* (Coleman, 1969). In both supernatant fluid and pellet fractions, all the amino acids in the protein were labelled. There was no evidence for the biosynthesis of purines or pyrimidines from glucose. When the protozoa were disrupted before incubation with $[\text{U-}^{14}\text{C}]\text{glucose}$, incorporation into the broken-cell pellet in the absence of antibiotic increased, suggesting that at least some of the bacteria in intact protozoa were not freely in contact with $[\text{U-}^{14}\text{C}]\text{glucose}$ in the medium.

A reciprocal plot of uptake of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ by *P. multivesiculatum* against glucose concentration (Fig. 1), showed a sharp discontinuity in the curve (at 12 mM) for both the complete protozoon and the broken-cell supernatant fluid, suggesting that there are two uptake systems. On the basis of arguments similar to those used previously (Coleman, 1967*a*, 1969), it is postulated that there is an 'active' system predominant at low concentrations of glucose and a passive one predominant at high concentrations.

Mechanisms of glucose uptake. During the incorporation of ^{14}C from 1 mM- $[\text{U-}^{14}\text{C}]\text{glucose}$, ^{14}C appeared sequentially in glucose 6-phosphate, glucose, maltose and glucose 1-phosphate. The specific activities (in counts $\text{min}^{-1} \mu\text{mol}^{-1}$) after 1 min incubation were: glucose 6-phosphate, 19400; glucose, 5500; and maltose, 3100. These suggested that the primary product of glucose uptake was glucose 6-phosphate which was then metabolized to form glucose and maltose: the amount of glucose 1-phosphate was too small to measure. There was no evidence for a greatly increased rate of incorporation into protozoal polysaccharide at high external glucose concentrations as was found with *Epi. ecaudatum caudatum* (Coleman & Laurie, 1976).

Metabolism of starch

To determine whether *P. multivesiculatum* synthesized protein from intact starch grains (which are probably its commonest source of food in the rumen), the protozoa were incubated with ^{14}C -labelled starch grains prepared as described by Coleman (1969); (0.22 % of the ^{14}C in these grains was present as protein in which all the amino acids were labelled). After incubation for 20 h in the presence or absence of ampicillin (600 $\mu\text{g ml}^{-1}$), or for 45 h in the presence of antibiotic with $[\text{U-}^{14}\text{C}]\text{starch}$ being added to the medium after 18 h, over 80 % of the ^{14}C initially present disappeared when a sample of the complete culture placed on a planchette was acidified. The proportions of the initial ^{14}C that were found in the protein fractions of the protozoa at the end of the experiment were (results for the broken-cell supernatant fluid in parentheses) 3.8 % (2.0 %) in the absence of antibiotic, and 0.9 % (0.23 %) or 2.1 % (0.49 %) when the starch was added initially or after 18 h in the presence of ampicillin. As these results are greater than the amount of ^{14}C -labelled protein added initially they suggest that the protozoa can synthesize protein from carbohydrate. In the protein from the broken-cell supernatant fluid from protozoa incubated with ampicillin, only aspartic acid, alanine, glutamic acid and serine were labelled, whereas in the other protein fractions all amino acids contained ^{14}C .

After incubating 780 protozoa ml^{-1} with soluble $[\text{U-}^{14}\text{C}]\text{starch}$ (100 μg ; 1 $\mu\text{Ci ml}^{-1}$) for 2 min, glucose 6-phosphate contained 690 counts min^{-1} (sp.act. 6000 counts $\text{min}^{-1} \mu\text{mol}^{-1}$); maltose, 670 counts min^{-1} (sp.act. 4100 counts $\text{min}^{-1} \mu\text{mol}^{-1}$); glucose, 110 counts min^{-1} (sp.act. 1970 counts $\text{min}^{-1} \mu\text{mol}^{-1}$); and glucose 1-phosphate, 650 counts min^{-1} (sp.act. is probably high as the amount of glucose 1-phosphate present was too small to measure).

Table 3. Comparison of the size and metabolic capabilities of three species of rumen ciliate protozoa

The different parts of this experiment were all carried out simultaneously with the same batch of organisms.

	<i>Ento. caudatum</i>	<i>Epi. ecaudatum</i> <i>caudatum</i> (ovine)	<i>P. multivesicu-</i> <i>latum</i> (grown without epidinia)	<i>P. multivesicu-</i> <i>latum</i> (grown with epidinia)
Size relative to <i>Ento. caudatum</i>				
Surface area	1	3.3	5.4	10.8
Volume	1	5.5	14.5	36
Uptake of 0.67 mM-[¹⁴ C]glucose in 1 h [μ mol (10 ⁶ protozoa) ⁻¹]	0.01	10	35	40
Uptake of ³⁵ S-labelled bacteria (10 ⁹ ml ⁻¹) in 3 h [bacteria protozoon ⁻¹]	1 270	2 100	26 600	37 000
Uptake of ¹⁴ C-labelled <i>Esch. coli</i> (10 ⁹ ml ⁻¹) in 3 h [bacteria protozoon ⁻¹]	1 030	1 460	18 000	175 000

These results suggest that starch was metabolized by phosphorolysis to glucose 1-phosphate followed by isomerization to glucose 6-phosphate, rather than by hydrolysis to maltose and glucose and phosphorylation of this to glucose 6-phosphate as was found for *Entodinium caudatum* (Coleman, 1969).

DISCUSSION

Polyplastron multivesiculatum, like *Entodinium* spp. and *Epidinium* spp. (Coleman, 1964, 1967b, 1972; Coleman & Laurie, 1974) engulfed a wide range of bacteria. Although there was considerable variation between the rates of uptake by these species, this variation was less when the comparison was made on the basis of the volume of medium cleared of bacteria rather than on the number of bacteria engulfed. Therefore, *P. multivesiculatum* probably passes a constant volume of medium in and out of its oesophagus each hour and does not change the rate of passage of medium with change in the bacterial species present. Any alteration in the rate at which bacteria were engulfed would then depend on variations in the ability of the protozoa to take up individual bacteria into vesicles in the cytoplasm. If it is assumed that the fastest rate of medium clearance found, i.e. $150 \times 10^6 \mu\text{m}^3 \text{h}^{-1}$ protozoon⁻¹ with an infinitely dilute suspension of *Streptococcus bovis* (Table 1), is the actual rate at which medium passes in and out of the oesophagus, then the lower rates found with other bacteria, especially at high population densities, show that the protozoa are usually inefficient at removing these bacteria from the medium. For example, with *Klebsiella aerogenes* at 10⁹ ml⁻¹, only 4 % of the bacteria are removed by the protozoa. The efficiency with which bacteria are removed also depends on the species and its population density. With *Butyrivibrio fibrisolvens*, the efficiency is high at low population densities but decreases with increasing density so that the total number of bacteria engulfed remains constant. However, with *Proteus mirabilis*, the efficiency remains constant with increasing bacterial population density resulting in a steady increase in the number of bacteria engulfed.

A comparative study of the metabolism of three important rumen protozoa (Table 3) indicates that *Entodinium caudatum* takes up glucose much more slowly than *Epi. ecaudatum caudatum* or *P. multivesiculatum* on the basis of volume or surface area. However, on a volume basis, *Ento. caudatum* takes up mixed rumen bacteria and *Esch. coli* at about the same rate as *P. multivesiculatum* (grown in absence of epidinia) but more rapidly than *Epi.*

ecaudatum caudatum. *Polyplastron multivesiculatum*, grown in the presence of epidinia, takes up *Esch. coli*, but not mixed rumen bacteria, much more rapidly than when grown in the absence of other protozoa. With low concentrations of amino acids, after allowing for variation between different amino acids, *P. multivesiculatum* took up amino acids 5 to 20 times as fast as either *Epi. ecaudatum caudatum* or *Ento. caudatum* (Coleman & Laurie, 1974).

The metabolic capabilities of *P. multivesiculatum* with respect to amino acid and purine and pyrimidine biosynthesis were similar to those of *Epi. ecaudatum caudatum* (Coleman & Laurie, 1974) except that about twice as much of the ^{14}C incorporated from $[\text{U-}^{14}\text{C}]\text{glucose}$ was found in the protein fractions, possibly suggesting a greater ability to synthesize amino acids.

Although the experiments reported above were made with washed suspensions of *P. multivesiculatum*, which may not behave in the same way as growing organisms, the results can be used to obtain information on the possible source of protein for protozoal growth. As the protozoa have associated bacteria that are found after sonication in the broken-cell pellet fraction, only incorporation into the broken-cell supernatant fluid will be considered. Each *P. multivesiculatum* grown in the presence of an *Epidinium* sp. contained 69 ng protein in this fraction and would therefore have to synthesize $2.9 \text{ ng protein h}^{-1}$ to divide each day. As each protozoon engulfed $12\,000$ mixed rumen bacteria h^{-1} (from a population of 10^9 ml^{-1}) and as sulphur from one third of these appeared in the protozoal broken-cell supernatant fluid, $0.72 \text{ ng protein h}^{-1}$ could come from this source. The uptake of an amino acid (from 0.1 mM solution) at 2 pmol h^{-1} could supply $3.6 \text{ ng protein h}^{-1}$ assuming that all amino acids are present in solution and that all are taken up at approximately the same rate. As 4 % of the glucose carbon taken up was found in the protein of the broken-cell supernatant fluid, an uptake of 40 pmol h^{-1} (from an 0.67 mM solution) could supply $0.3 \text{ ng protein h}^{-1}$. Thus rumen bacteria and amino acids could, at the levels present in the rumen, supply sufficient protein to enable the protozoon to divide every 16 h. However, *P. multivesiculatum* in culture also engulfs at least one epidinium per day; this would supply a further 20 ng protein and enable the protozoon to divide even more frequently. *Polyplastron multivesiculatum* grown in the absence of other protozoa would require $1.6 \text{ ng protein h}^{-1}$ to divide once each day but by similar arguments to those used above, could obtain 1.97 ng from bacteria and amino acids and divide every 20 h. These calculations show that whereas *Ento. caudatum* obtains the amino acids required for growth solely by engulfment and digestion of bacteria, free amino acids in the medium are an important source of these compounds for *P. multivesiculatum*.

We wish to thank Dr P. Kemp for inserting permanent rumen cannulae into the sheep used to provide rumen fluid, and Miss J. E. Bailey for valuable technical assistance.

REFERENCES

- COLEMAN, G. S. (1964). The metabolism of *Escherichia coli* by *Entodinium caudatum*. *Journal of General Microbiology* **37**, 209–223.
COLEMAN, G. S. (1967*a*). The metabolism of free amino acids by washed suspensions of the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology* **47**, 433–447.
COLEMAN, G. S. (1967*b*). The metabolism of the amino acids of *Escherichia coli* and other bacteria by the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology* **47**, 449–464.
COLEMAN, G. S. (1968). The metabolism of bacterial nucleic acid and of free components of nucleic acid by the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology* **54**, 83–96.
COLEMAN, G. S. (1969). The metabolism of starch, maltose, glucose and some other sugars by the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology* **57**, 303–332.

- COLEMAN, G. S. (1972). The metabolism of starch, glucose, amino acids, purines, pyrimidines and bacteria by the rumen ciliate *Entodinium simplex*. *Journal of General Microbiology* **71**, 117-131.
- COLEMAN, G. S. & HALL, F. J. (1969). Electron microscopy of the rumen ciliate *Entodinium caudatum* with special reference to the engulfment of bacteria and other particulate matter. *Tissue and Cell* **1**, 607-618.
- COLEMAN, G. S. & LAURIE, J. I. (1974). The metabolism of starch, glucose, amino acids, purines, pyrimidines and bacteria by three *Epidinium* spp. isolated from the rumen. *Journal of General Microbiology* **85**, 244-256.
- COLEMAN, G. S. & LAURIE, J. I. (1976). The uptake and metabolism of glucose, maltose and starch by the rumen ciliate *Epidinium ecaudatum caudatum*. *Journal of General Microbiology* **95**, 364-374.
- COLEMAN, G. S., DAVIES, J. I. & CASH, M. A. (1972). The cultivation of the rumen ciliates *Epidinium ecaudatum caudatum* and *Polyplastron multivesiculatum* in vitro. *Journal of General Microbiology* **73**, 509-521.
- HOBSON, P. N. (1969). Rumen bacteria. *Methods in Microbiology* **3B**, 133-149.