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Effect of Absence of Ciliate Protozoa From the Rumen on Microbial Activity and Growth of Lambs

A. R. ABOU AKKADA AND K. EL-SHAZLY

Faculty of Agriculture, Alexandria University, Alexandria, Egypt

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ABSTRACT

ABOU AKKADA, A. R. (Alexandria University, Alexandria, Egypt), AND K. EL-SHAZLY. Effect of absence of ciliate protozoa from the rumen on microbial activity and growth of lambs. Appl. Microbiol. **12**:384–390. 1964.—A survey of the components of the rumen ciliate population in a series of adult sheep, raised in the Faculty of Agriculture, University of Alexandria, has shown that a mixture of *Entodinium*, *Isotricha*, *Ophryoscolex*, *Diplodinium*, and *Polyplastron* species was found in the rumen contents of Egyptian sheep; no *Epidinium* and a negligible number of *Dasytricha ruminantium* were also observed. The microbial population, reducing sugars, ammonia, volatile fatty acids (VFA) production, and growth rate of 14 lambs inoculated with whole rumen contents from a mature sheep were compared over a 6-month period with those of 13 lambs maintained under the same conditions, except that they were strictly isolated from other ruminants. Certain large oval organisms and large numbers of flagellates and *Oscillospira* were frequently observed in the rumen contents of the isolated lambs. The reducing sugars, ammonia, and VFA levels, measured before and at intervals after feeding, in the inoculated lambs showed a pronounced rise above the values found in the ciliate-free animals. The propionic acid-acetic acid ratio in the rumen contents of the faunated lambs was considerably higher than in the nonfaunated controls. The inoculated lambs grew faster than the isolated lambs. Differences in weight gain which ranged from 15 to 17% were statistically significant. The inoculated animals impressed the observers by their good appearance which was superior to that of the ciliate-free lambs. It was, therefore, concluded that the rumen ciliate protozoa are essential for the metabolism and growth of young lambs.

In general, there is an immense population of ciliate protozoa in the rumen of domestic ruminants. Their number, according to Hungate (1950), may exceed one million per gram of rumen contents and their mass roughly equals that of the bacteria. In vitro studies (Heald and Oxford, 1953; Gutierrez, 1955; Howard, 1959*a*, b; Abou Akkada and Howard, 1960) indicated that holotrich rumen protozoa can metabolize soluble carbohydrates to produce volatile fatty acids (VFA), gas, and storage polysaccharides. Hungate (1942, 1943) was the first to show, and Abou Akkada, Eadie, and Howard (1963) confirmed, that extracts from some members of rumen oligotrich protozoa contained an active cellulase. Suspensions of the rumen protozoa *Isotricha* spp. (Abou Akkada and Howard, 1961) and *Ophryoscolex caudatus* (Williams et al., 1961) readily decomposed pectic substances; extracts of *Isotricha* spp. and *Polyplastron multivesiculatum* contained appreciable concentrations of pectin esterase and polygalacturonase (Abou Akkada and Howard, 1961; Abou Akkada et al., 1963). Hemicelluloses are rapidly hydrolyzed by extracts from the rumen protozoa *Epidinium ecaudatum* (Baily, Clarke, and Wright, 1962) and *P. multivesiculatum* (Abou Akkada et al., 1963). Several proteins are converted into peptides and amino acids by suspensions of *O. caudatus* and *Entodinium caudatum* isolated from the rumen of sheep and cows (Williams et al., 1961; Abou Akkada and Howard, 1962). Biosynthesis of some amino acids by these organisms was recently demonstrated (Williams et al., 1961; Coleman, 1963). Wright (1959, 1961) showed that ciliate protozoa are probably involved in hydrogenating double bonds of the rumen unsaturated fatty acids, and it has been demonstrated (Oxford, 1958) that *Epidinium ecaudatum* obtained from the rumen of a cow fed on clover can ingest chloroplasts which contain much of the lipids of leaves. The view, therefore, has long been held that the rumen ciliate protozoa may contribute up to 20% of their host's nutritional requirements under favorable conditions (Hungate, 1955; Oxford, 1955; Gutierrez, Davis, and Warwick, 1960).

However, on the basis of experiments carried out with goats, sheep, and calves maintained under defaunation conditions, it was suggested that the absence of protozoa did not cause any ill effects to the animals (Becker, Schultz, and Emmerson, 1930; Pounden and Hibbs, 1950; Bryant and Small, 1960; Eadie, 1962*a*). Hungate (1950) postulated that the role of rumen protozoa might be taken over in their absence by the bacteria so that the host does not suffer. On the other hand, although Pounden and Hibbs (1950) and Eadie (1962*a*) observed no striking ill effects on nonfaunated calves, there was some indication that they did not thrive so well as the faunated. Moreover, growing lambs containing rumen protozoa gained slightly less weight over a 3-month period than did nonfaunated controls (Becker and Everett, 1930). Because almost all the previous studies were only descriptive and the number of animals used in each comparison was too small to justify statistical conclusions, the present investigation was undertaken to determine quantitatively the extent to which rumen protozoa contribute to the growth of lambs. A report on the establishment and

components of the rumen protozoal population in a series of Egyptian sheep is also included.

MATERIALS AND METHODS

Animals. The 26 Barky and 4 crossbred (Barky × Rahmany) lambs used were removed from the dams as they were born, and were housed in a separate pen. Almost all animals were born within a period of 8 days and were, therefore, very similar in their average initial weight. The lambs were allowed to receive colostrum twice daily for 3 days, during which precautions were taken to prevent any direct mouth-to-mouth contact between the animals and their mothers. On the fourth day, the lambs were separated from their mothers and were bottle-fed whole milk. Berseem (*Trifolium alexandrinum*) was given ad lib. The lambs were handled and fed only by two persons, who had no contact with the rest of the farm flock. At 5 weeks, stomach-tube samples were withdrawn from all lambs to examine for protozoa. Animals proved to be ciliate-free. They were then divided into two groups containing equal numbers of each sex and breed, similar in average initial weight. One group remained in an isolated pen with high partitions, under strict precautions to prevent any contact with ruminants. Lambs of the other group were inoculated with rumen protozoa and kept in a separate pen. From the seventh week, the general performance and weekly weight of the isolated ciliate-free lambs and their inoculated controls were compared. Table 1 shows various feeding regimens under which the two groups were kept throughout the period of the experiment.

Inoculation and sampling. Whole rumen liquor from a mature sheep on Berseem (*T. alexandrinum*) was used as inoculum. Each lamb was drenched with about 60 to 80 ml of the rumen contents immediately after the rumen liquor was withdrawn from the sheep.

Polyethylene stomach tubes were used for obtaining rumen samples from the lambs. Samples from the isolated lambs were taken frequently after the morning feed to ensure the exclusion of the ciliate protozoa from them. Samples of rumen liquor from six isolated lambs and six of their inoculated controls were also obtained for the estimation of reducing sugars, ammonia nitrogen, and total VFA. Samples were withdrawn from four lambs of each group (experiment 1) when animals were on concentrate mixture 1 (Table 1). The rumen contents of the other lambs of each group (experiment 2) were also sampled, when the animals were given cottonseed cake-rice bran mixture (Table 1). The first sample was collected at 8:45 AM, before introducing the ration, and the others were at 1, 2, 4, and 6 hr after feeding.

Microscopic examinations were made on wet preparations from well-mixed samples to give an approximate indication of types and numbers of the ciliate protozoa in the rumen liquor. The examinations were repeated on formalin-fixed material to assess the protozoal population accurately.

Methods of analysis. Rumen liquor used in all estimations was deproteinized by mixing the sample (10 ml) with 0.1 N HCl (10 ml) in a 50-ml volumetric flask and filling to the mark with distilled water (Warner, 1956).

Ammonia N in 1-ml portions of the deproteinized samples was estimated in Conway (1957) units.

Reducing sugars were determined in the deproteinized rumen liquor by Nelson's (1944) method. In some experiments, the anthrone method (Abou Akkada and Howard, 1960) was also used.

Total and individual VFA were estimated as described by el-Shazly, Abou Akkada, and Naga (1963).

Identification of the ciliates in the rumen liquor of lambs and adult sheep was carried out as described by Eadie (1962*b*).

The results were analyzed statistically according to Snedecor (1956).

RESULTS

Microscopic examinations were made on fresh and formalin-fixed samples from a series of Barky and Rahmany sheep, chosen from the stock of the Faculty of Agriculture, Alexandria University, and fed on Berseem (*T. alexandrinum*). It was found that the ciliate population of these sheep consisted mainly of *Entodinium*, *Isotricha*, and *Ophryoscolex* spp.; fair numbers of *Polyplastron* and *Diplodinium* spp. were also observed. *Dasytricha ruminantium* was invariably absent, or was present in very small numbers. No *Epidinium* spp. were ever seen in rumen contents from Egyptian sheep. The ciliate

TABLE 1. *Rations of isolated and inoculated lambs throughout the experiment**

Date	Rations given to each lamb
2/28/63	Colostrum for 3 days
3/11/63	600 ml of whole milk fed twice daily + Berseem (*Trifolium alexandrinum*) ad lib
6/2/63	600 ml of whole milk and 150 g of concentrate mixture 1 + Berseem ad lib
6/14/63	300 ml of whole milk and 200 g of concentrate mixture 1 + Berseem hay ad lib
6/21/63	250 g of concentrate mixture 1 fed at 8 AM + 0.5 kg of Berseem hay at 3 PM
8/4/63	250 g of cottonseed cake-rice bran mixture (1:1) at 9 AM + 0.5 kg of Berseem hay at 3 PM
8/20/63	0.5 kg of cottonseed cake-rice bran mixture + 0.5 kg of Berseem hay
9/4/63	0.5 kg and 2 kg, respectively, of cottonseed cake-rice bran mixture and of chopped green maize in the morning + 0.5 kg of Berseem hay
10/19/63	0.5 kg of cottonseed cake-rice bran mixture at 9 AM + 1.0 kg of Berseem hay at 3 PM

* Almost all lambs were born within the period from 2/8/63 to 2/28/63. Concentrate mixture 1 consisted of maize grains, 35 parts; barley, 10 parts; molasses, 8 parts; linseed-oil meal, 15 parts; horse beans (*Vicia faba*), 30 parts; calcium carbonate, 1.5 parts; and mineral mixture, 0.5 part.

population of Barky sheep was remarkably similar to that of Rahmany sheep.

Microscopic examinations also indicated that no ciliates appeared in the isolated lambs until they were inoculated. The time after which the ciliate protozoa were observed in the rumen contents of inoculated lambs is shown in Table 2. It is evident that an active mixed population of protozoa had developed in the isolated lambs as early as 6 to 11 days after inoculation. Although, in almost all the lambs, *Entodinium* and *Isotricha* organisms were the first to develop, yet a large number of *Entodinium* spp. tended to be more rapidly established in lambs than were the *Isotricha* spp.; small numbers of *Entodinium* spp. were seen in lambs 960 and 996 by 3 days from inoculation. In most of the lambs *Isotricha* spp. became established before *Ophryoscolex* spp.; in lambs 984, 997, and 998, appreciable numbers of both types were observed at the same time. *Diplodinium* and *Polyplastron* species were late comers. *Dasytricha ruminantium* were not observed in several lambs.

Direct observations on stomach-tube samples showed some distinct differences in the microbial population of the isolated and inoculated lambs. The numbers of *Oscillospira* in the isolated lambs were remarkably higher than those in the inoculated animals. Great numbers of the large oval organism described by Eadie (1962*a*) were observed in the isolated lambs; the rumen contents of inoculated lambs did not contain this organism, although all lambs were on the same ration (Table 1). The organism very frequently showed some visible internal structures and signs of cell division. It was seen in the rumen contents of the isolated lambs as early as 4 to 6 weeks and it stayed as long as 6 months. Exceptionally high numbers of actively motile flagellates were observed in the rumen contents of ciliate-free lambs. These organisms were seen when it was first possible to sample the lambs (4 to 6 weeks old).

TABLE 2. *Time of first establishment of ciliate protozoa in lambs inoculated after a period of isolation*

Lamb no.	Days after inoculation	Rumen ciliate protozoa*					
		Entodinium	*Isotricha*	*Ophryoscolex*	*Polyplastron*	*Diplodinium*	*Dasytricha*
960	6	++	−	−	−	−	−
	11	+++	+++	++	+	+	−
961	6	++	+	−	−	−	−
	11	+++	++	+	+	+	−
965	6	++	−	−	−−	−	−
	11	+++	++	+	+	++	+
970	6	+++	+	−	−	−	−
	11	+++	++	++	+	+	−
984	6	+++	++	++	+	++	−
	11	+++	+++	++	+	++	−
992	6	++	+	−	−	−	−
	11	+++	++	−	+	++	−
996	6	+++	+	−	−	−	−
	11	+++	++	++	+	+	+
997	6	+++	++	++	−	−	−
	11	+++	++	++	+	+	+
998	6	+++	+	++	−	−	−
	11	+++	++	++	−	++	−
1001	6	++	−	−	−	−	−
	11	+++	++	−	−	+	−
1010	6	++	−	+	+	+	−
	11	+++	−	++	+	++	−

* The original inoculum contained large numbers of *Entodinium* and *Isotricha*, and appreciable numbers of *Ophryscolex*, *Polyplastron*, and *Diplodinium*; small numbers of *Dasytricha* were also present. Symbols: −, +, ++, and +++; protozoa not observed, present in small, appreciable, and large numbers, respectively.

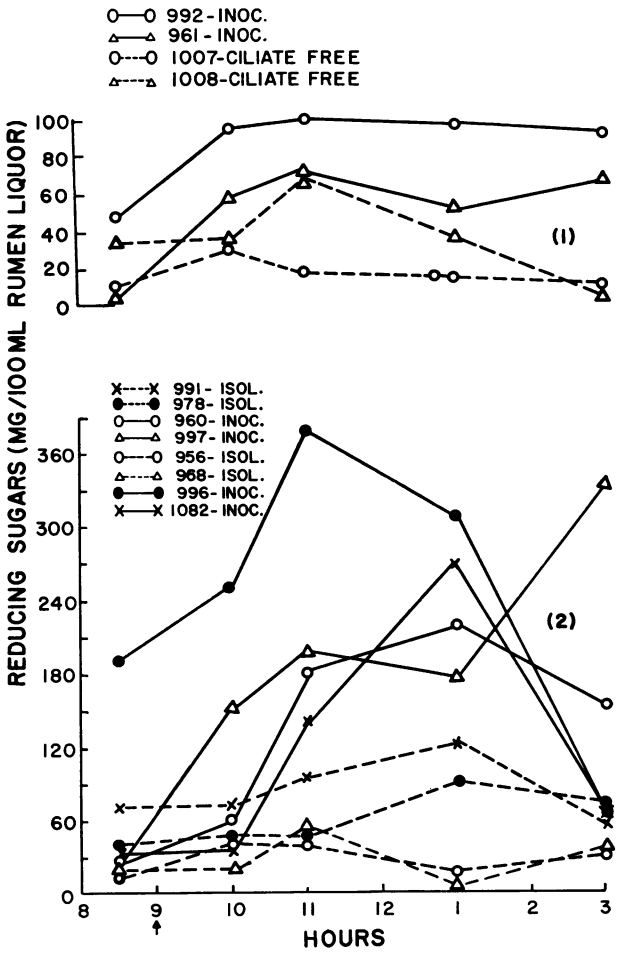

FIG. 1. *Variation in the concentration of reducing sugars. Lambs were fed cottonseed cake-rice bran mixture.*

FIG. 2. *Variation in the concentration of reducing sugars. Lambs received concentrate mixture 1. Lambs 960, 997, 956, and 968 were sampled on 7-12-1963; lambs 996, 1082, 978, and 991 were sampled on 7-31-1963.*

The concentrations of reducing sugars determined in the rumen liquor just before feeding and at four different intervals after feeding are shown in Fig. 1 and 2. Under the same feeding regimen, reducing sugar concentration in the inoculated lambs was substantially higher than in the ciliate-free animals. Also, in both isolated and inoculated lambs, the concentrate mixture 1 gave rise to concentrations of reducing sugars which were above values produced by the cottonseed cake-rice bran mixture. The curve of reducing sugars for most of the lambs started at a very low level just before feeding and then rose until it reached a maximum 2 hr after feeding.

In Fig. 3 and 4 is shown the ammonia N concentration in milligrams per 100 ml of the rumen liquor, estimated for each animal at five intervals. It is obvious that, when the lambs were eating the same rations, ammonia N levels were greater in inoculated lambs than in their isolated controls. In inoculated lambs, ammonia production showed a fairly sharp rise after feeding and was greatest between 2 and 4 hr after feeding. On the other hand, the rise in ammonia curve was slow and slight in the ciliate-free lambs. There were also fair differences in ammonia production between concentrate mixture 1 and cottonseed cake-rice bran mixtures.

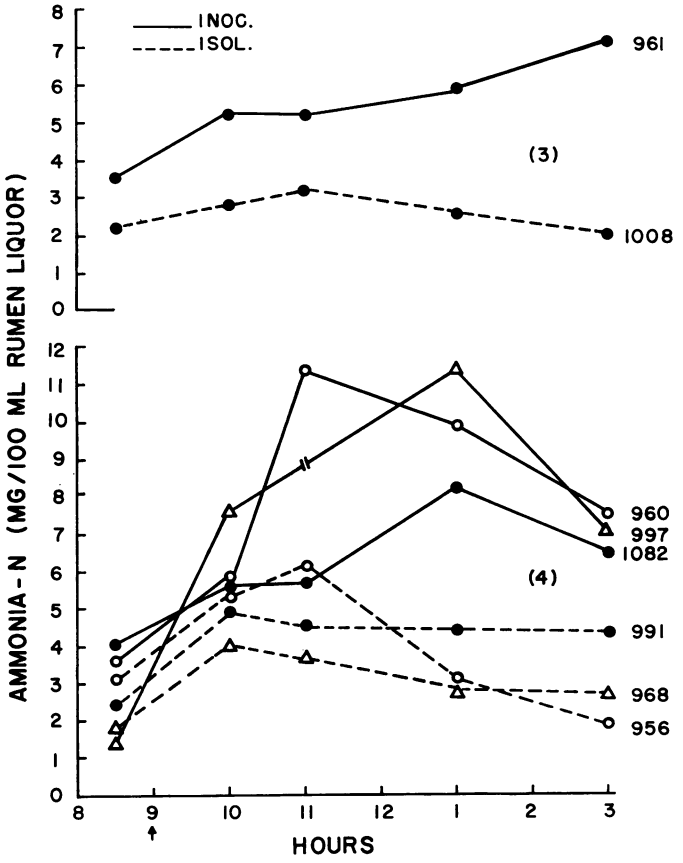

FIG. 3. *Variation in the concentration of ammonia N in the rumen liquor of lambs receiving cottonseed cake-rice bran mixture.*

FIG. 4. *Variation in the concentration of ammonia N in the rumen liquor of lambs receiving concentrate mixture 1.*

Experiments on VFA concentrations in milliequivalents per 100 ml of the rumen liquor (Fig. 5 and 6) showed a pronounced increase in values of the inoculated lambs over those of the isolated controls. VFA levels were particularly low in postfeeding samples and reached a maximum approximately 2 to 4 hr after feeding; thereafter, they declined. Concentrate mixture 1 was associated with significantly higher rumen VFA levels in most experiments (Fig. 5 and 6). The VFA, upon chromatographic analysis, were shown to consist of formic, acetic, propionic, and butyric acids (Table 3). The acetic acid together with propionic acid accounted for an average of 95 and 97% of the total VFA, respectively, in the inoculated and isolated lambs. Small concentrations of butyric acid were found in the rumen liquor from isolated animals, whereas the rumen contents of inoculated lambs were devoid of this acid. The average propionic acid-acetic acid ratio in the rumen liquor from the isolated and inoculated lambs was 0.265 and 0.393, respectively.

The number of lambs in the isolated and inoculated group, their average initial age and weight, and their average weight gain are presented in Table 4. The period during which the two groups were compared (168 days) was divided into three subperiods, calculated from the time the experiment started, for measuring the average gain in each period. It was hoped to find out at what stage the differences between the two groups were ob-

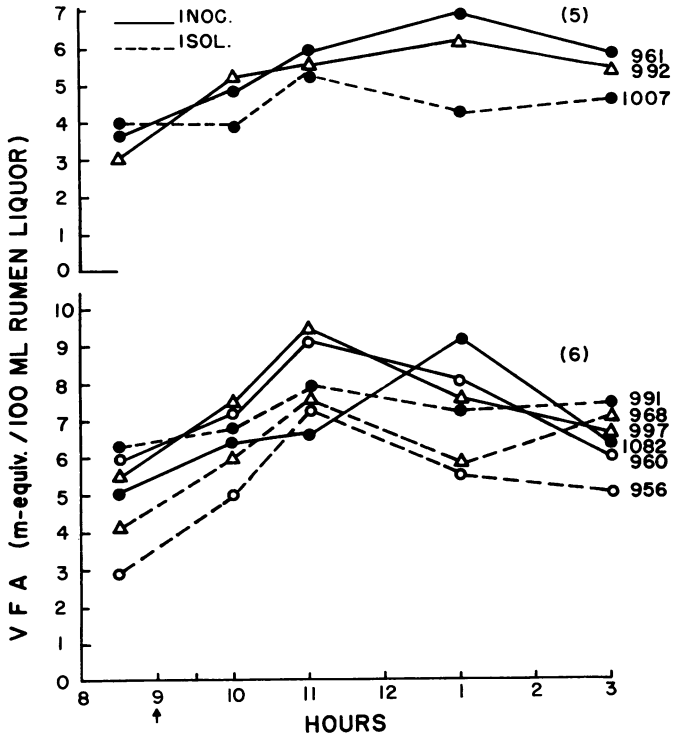

FIG. 5. *Variation in the concentration of total volatile fatty acids in the rumen liquor of lambs receiving cottonseed cake-rice bran mixture.*

FIG. 6. *Variation in the concentration of total volatile fatty acids in the rumen liquor of lambs receiving concentrate mixture 1.*

vious. It can be seen that the inoculated lambs grew faster than the protozoa-free controls in the three subperiods. There was a difference of 24.0, 15.7, and 16.5%, respectively, in the first (49 days), second (119 days), and third (168) period, although extra care was directed to the management of the isolated lambs. It seems that the growth in the inoculated lambs was slightly checked in the period from the 49th to 119th days. It is noteworthy that in this period the inoculated animals were given two doses of Minel (liver fluke remedy), which contained 59.02% phenothiozine, 39.34% hexochloroethane, 0.82% copper sulfate, and 0.82% cobalt sulfate, as there was some contact between these lambs and the neighboring animals which grazed daily in the field. The remedy was administered orally in doses of 10 g each. Minel caused the animals to be under strain for 3 weeks, and thereafter they gradually recovered. Isolated lambs were given only one dose of Minel, because the possibility of being infected with the fluke was very small, and it was thought that an extra dose might endanger their lives as judged by their appearance after the first dose had been introduced. Analysis of variance showed that the increase in weight gain in the inoculated lambs over the ciliate-free controls was significant in the first two periods; the overall increase in the last period (168 days) was highly significant. Moreover, the inoculated lambs impressed the observers by their superiority in general appearance to the ciliate-free animals.

TABLE 3. *Individual volatile fatty acids produced in the rumen of isolated and inoculated lambs*

Animals	Expt no.*	Volatile fatty acids†			
		Butyric acid	Propionic acid	Acetic acid	Formic acid
Isolated	1a	1.2	15.8	81.5	1.5
	1b	T	25.6	71.2	3.2
	1c	T	21.9	75.0	3.1
	2	7.6	14.9	77.5	—
Inoculated	1a	—	21.4	74.0	4.6
	1b	—	25.4	67.0	7.6
	1c	—	28.0	64.5	7.5
	2	—	32.5	67.5	—

* In experiment 1 the lambs were fed on concentrate mixture 1; cottonseed cake-rice bran mixture was offered to lambs in experiment 2; a, b, and c refer to lambs used as source of rumen liquor in experiment 1. Samples from lambs a of both inoculated and isolated lambs were obtained on 7-12-1963; whereas lambs b and c of both groups were sampled on 7-31-1963.

† Results are expressed as percentage of total volatile fatty acids. T = present in traces; — = not present.

TABLE 4. *Rate of growth of isolated and inoculated lambs*

Item	Isolated group	Inoculated group
Number of lambs	13[a]	16
Average initial age (days)	51.00 ± 1.5[b]	48.00 ± 3.5
Average initial weight (kg)	7.70 ± 0.30	8.30 ± 0.55
Average gain (kg) after 49 days	4.73 ± 0.26	5.92*[c] ± 0.53
Average gain (kg) after 119 days	11.40 ± 0.22	13.20* ± 0.69
Average gain (kg) after 168 days	15.30 ± 0.44	17.80** ± 0.68

[a] The isolated group started with 14 lambs, but lamb 980 died 49 days later; thus, data concerning its growth and that of a corresponding lamb in the inoculated group were excluded.

[b] Standard error.

[c] Single asterisk = significant at the 5% level; double asterisk = significant at the 1% level.

DISCUSSION

The microscopic observations suggest that there is a regional difference in the genera of ciliate protozoa inhabiting the rumen of sheep. Although *D. ruminantium* is commonly seen in Scottish sheep (Eadie, 1962*a*, *b*), very few or none of these organisms was observed in the rumen contents from the Egyptian sheep. Similarly, the protozoal population of the Australian sheep consisted largely of small oligotrichous forms with occasional holotrichs (Moir and Summers, 1957; Purser and Moir, 1959). This regional difference does not appear to be due solely to the change in rations offered to the animals. Although *Epidinium* was fairly abundant and *Ophryoscolex* was absent in New Zealand sheep fed on *Trifolium pratense* (Oxford, 1958), the reverse was true in Egyptian sheep given *T. alexandrinum*. This difference could be explained by the conclusion of Eadie (1962*b*) that the factors which govern the establishment of a rumen ciliate fauna are not only the alterations in rumen conditions due to change in the diet but also those due to antagonism between ciliate species. Apart from the last mentioned differences, the genera which make up the protozoal population of Egyptian sheep resemble those found in the rumen of other sheep (Eadie, 1962*a*), namely, *Isotricha*, *Entodinium*, *Polyplastron*, and *Diplodinium* species.

When the isolated ciliate-free lambs were inoculated with rumen liquor from adult sheep, a thriving mixed population became established within 6 to 11 days. This is in accord with results of other workers (Bryant and Small, 1960; Eadie, 1962*a*), showing that rumen inoculation or mouth-to-mouth contact with adult ruminants is necessary before ciliate protozoa are established. The present results also suggest that there are some differences in the microbial population between the inoculated lambs and the isolated controls. These findings are in agreement with those of Eadie (1962*a*), who observed considerable numbers of *Oscillospira* and a large oval organism in the isolated lambs. Moreover, a numerous and active population of flagellates was frequently observed in the ciliate-free animals. No large oval organisms, and reduced numbers of *Oscillospira* and flagellates, were observed in the rumen contents of the faunated lambs.

The most striking feature of the present results is the undoubted ability of the faunated lambs to metabolize

the food constituents more efficiently than do the ciliate-free lambs. This is best indicated by the greater growth observed in the former group. Reducing sugars, ammonia, and total VFA concentrations were remarkably higher in the rumen of inoculated animals, which correlates well with growth data. These findings have revived the conclusions put forward by previous workers (Becker, 1932; Becker et al., 1930; Mangold, 1933). Becker (1932) found that when faunated sheep were fed on ground barley over 80% of starch grains were ingested and then converted into protozoal glycogen within 6 hr, whereas defaunated sheep on a similar diet appeared to be bloated. Mangold (1933) stated that the proportion of food protein metabolized by rumen fauna and subsequently digested by the host is considerable. Australian workers (Weller, Gray, and Pilgrim, 1958) concluded that, of the rumen nitrogen, 46% was bacterial and 21% was protozoal. Moreover, Hungate (1955) suggested that 20% of the fermentation acids produced in the rumen are contributed by the protozoa. Other aspects of the metabolism of faunated and isolated lambs, such as the determination of blood hemoglobin levels and digestibility experiments, are being studied in this laboratory, to throw more light on the role played by rumen protozoa in the digestive economy of the host animals.

There is now no doubt that the VFA produced in the rumen contribute a major part of the total energy absorbed from the alimentary tract in the ruminants (Blaxter, 1961). The individual volatile fatty acids differ in the extent to which they promote synthesis of depot fat (Blaxter, 1961; Sutherland, 1963). When sheep receiving a constant ration were given VFA, it was shown that the calorimetric efficiency of fat synthesis from propionic acid was higher than from acetic acid (Blaxter, 1961). Our chromatographic analysis indicated that propionic acid accounted for an average of 27 and 19% of the total VFA formed in the rumen of faunated and isolated lambs, respectively (Table 3). The average propionic acid-acetic acid ratio was 0.393 for the inoculated lambs and 0.265 for the ciliate-free animals. It is not surprising, therefore, that the inoculated lambs had laid down more depot fat and grew faster than the isolated lambs (Table 4) throughout the experiment which lasted for 6 months. Differences between animals with and without ciliate protozoa, which ranged from 15 to 17%, were statistically significant. These results contrast with the observation that the absence of the ciliate protozoa did not have any striking effect on the animals' growth, health, or general performance (Becker et al., 1930; Pounden and Hibbs, 1950; Bryant and Small, 1960; Eadie, 1962*a*). Two of the last-mentioned experiments (Bryant and Small, 1960; Eadie, 1962*a*) were essentially designed to obtain information on the establishment of rumen protozoa, and in all of them the number of isolated animals raised for each comparison was too small to allow any statistical analysis or to demonstrate the real differences between the faunated and nonfaunated animals. Individual variations between animals, which could not be accounted for by difference in treatments, were noted by Pounden and Hibbs (1950), Bryant and Small (1960), and Eadie (1962*a*), thus stressing the importance of basing conclusions on a sufficiently large number of isolated animals. We have been fortunate in that 30 lambs born within a period of 8 days and with similar initial weights were at our disposal for an experiment lasting for 8 months. The results could be analyzed statistically after several intervals. Moreover, although the previous authors did not observe drastic ill effects on nonfaunated animals, there was an indication that they were not in as good condition as the faunated animals (Pounden and Hibbs, 1950; Eadie, 1962*a*). The ciliate-free animals had a rougher coat and a pot-bellied appearance. Hibbs and Pounden (1948) also observed differences in the blood plasma ascorbic acid levels between young inoculated and control calves on milk and hay ration. It is not possible to ascribe any of the favorable effects of inoculation to the bacteria present in the small (60 to 80 ml) inoculum obtained from the adult animal, because the groups of bacteria in the ciliate-free lambs made up an "adult type" population which is remarkably similar to those found in the inoculated controls (Bryant and Small, 1960; Eadie, 1962*a*; Abou Akkada and Blackburn, 1963).

More extensive experiments are being carried out in this laboratory to determine whether the significant differences between the faunated and nonfaunated lambs will continue to exist as the animals grow older. If they do, then the authors will be in a position to invalidate the 30-year-old conclusion of Becker and his colleagues that rumen ciliate protozoa are commensals and are not essential for the metabolism of the host animals. Our results, so far, indicate that these protozoa confer substantial benefits upon the growing lambs, even under moderately favorable conditions.

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