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Studies on the Alimentary Tract of Merino Sheep in South Africa. VI.—The Rôle of Infusoria in Ruminal Digestion with some remarks on Ruminal Bacteria.

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Introduction.

The process of digestion in the ruminant animal consists of mechanical, physical and chemical factors. The mechanical aspect has received due attention by numerous workers e.g. Wester (1926), Alvarez (1928), Bergman and Dukes (1925), Schalk and Amadon (1928), Trautmann and Schmitt (1933), Trautmann (1933), Brüggemann (1935), Krzywanek and Quast (1936). Quin and van der Wath (1938), and several others, have described the physiology of the ruminant stomach and the motility of the rumen in great detail.

The chemical and physical digestion in the forestomachs is, however, not yet understood, nor are the further processes of digestion and absorption in the abomasum and intestines known. Assumptions are freely based on what is known about the human animals with simple stomachs, but in the ruminant, anatomical differences and the differences in the type of diet, no doubt modify, or even completely alter the mode of digestion.

The composition of the predigested food passing from the rumen to the abomasum and intestines it totally unknown. The way in which cellulose is broken down, and its products of fermentation, are still a subject of speculation. Consequently, although the form in which the foodstuff is taken in is known, it has thus far been impossible to ascertain the chemical state in which this foodstuff ultimately enters the abomasum from the forestomachs, and also how this food is prepared for its final digestion in the abomasum and intestines.

The most important changes are due to the fermentative action of enzymes, both in the plants themselves, and especially those elaborated by the rich microfauna and flora in the rumen.

In fact there are indications that if these microorganisms of the rumen are destroyed, the host animal perishes soon after, so that a form of symbiosis exists between host and micro-organisms. It is the significance of these organisms which has stimulated the authors to attempt the elucidation of some of their digestive functions. For this purpose, merino sheep with closed permanent ruminal fistulae were used, as described by Quin and van der Wath (1938) while in some cases, material was withdrawn by stomach tube.

Previous work on this subject was carried out by one of four methods:—

(1) Material withdrawn from the rumen by stomach tube.

(2) Material obtained at the abbatoir.

(3) *In vitro* cultures (Westphal 1934).

(4) Digestion trials (Becker, Schulz and Emmerson 1930).

The micro-organisms studied by the authors comprised: (1) Infusoria, (2) Bacteria. In this paper, only the infusoria will be considered, but some remarks on bacteria are added where it was thought necessary to the elucidation of the infusorial function. A second paper dealing more particularly with ruminal bacteria, will be published in due course.

Literature.

Ever since the discovery of ruminal infusoria, by Gruby and Delafond in 1843, these organisms have interested research workers in a twofold way. Firstly as a source of complicated morphological and evolutionary studies (Dogiel, 1927) and secondly, as a biological problem.

Gruby and Delafond (1843), Stein (1858), Fiorentini (1889, 1890), Eberlein (1895), Crawly (1923), Dogiel (1921-1927) and many others have laboured incessantly to describe and classify the family Ophryoscolecidae. Dogiel, in his "Monograph" (1927) gave a final classification and description of all the known species and forms. Gruby and Delafond, Eberlein and Schuberg (1888) all expressed the opinion that the Ophryoscolecidae may have some biological significance. Ferber (1925, 1929), held that these organisms convert plant proteins into easily digestible animal protein, namely that of their own bodies, and that they serve as an important source of animal protein to their herbivorous hosts. According to Ferber, a sheep with 3 Kgm. rumen ingesta, and an infusorial population of 900 per cubic millimetre received ·327 gram of protein daily from its infusoria. If one considers that approximately half of this protein is of bacterial origin, it means that a sheep receives only about ·16 grams of infusorial protein over a period of 24 hours. This is insignificant, as the maintenance requirements of adult sheep of 50 Kgm. body weight are approximately 26 grams digestible protein daily (Smuts and Marais, 1938).

Further possible rôles attributed to the ruminal infusoria deserve mention.

(1) They are harmless commensals.

Biederman (1911), Scheunert (1924), Scheunert and Schieblich (1927) and others maintain that these organisms do neither harm nor good to their hosts and that they are merely found in the rumen because it is a favourable medium for them to live in. In 1929 Becker, Schulz and Emmerson came to a similar conclusion.

(2) They assist in the digestion of cellulose.

Since their discovery it has been thought that infusoria played a part in the digestion of cellulose in the rumen. Schuberg and Eberlein noted the ingestion of plant material by infusoria and concluded that the organisms could digest cellulose. Eberlein also noted the disintegration of plant material within the organisms and the expulsion of detritus from the anus. Dogiel and Federowa-Winogradowa (1925) observed that cellulose particles did not always leave the anus as detritus but that large particles were sometimes extruded morphologically unchanged; they suggested that these particles may have undergone a preliminary chemical digestion.

Mangold (1927) believes that cellulose digestion occurs within the infusoria by means of similarly ingested cellulose digesting bacteria.

In 1930 Becker, Schulz and Emmerson conducted digestion trials by using goats with and without infusoria, and concluded that cellulose digestion in the host is neither due to, nor materially assisted by the infusoria. Unfortunately the diet of these goats contained a high proportion of grain which naturally attracts infusoria so that the cellulose in the diet can be considered to have received very little attention from the infusoria. These organisms definitely prefer starch to cellulose. Appreciable quantities of cellulose are ingested by the infusoria only when grain is not present in the rumen.

(3) They assist in the digestion of starch.

Trier (1926) and Westphal (1934) drew attention to the relationship between infusoria and the digestion of starch. Westphal did not assign any significance to the digestion of starch by infusoria and from the results of his *in vitro* work he concluded that they were only commensals particularly dependent on starch.

(4) They are mechanical and physical aids in digestion. Bundle (1895), Braune (1913), Scheunert (1924) and Scheunert and Schieblich (1927) considered that these micro-organisms were purely of mechanical and physical importance to their host and assisted in the soaking, macerating and mixing of the rumen contents.

(5) Infusoria are injurious parasites.

Apart from the view held by Zürn (1887) that infusoria are injurious parasites as their presence may lead to a catarrh of the alimentary tract, no other workers have suggested a similar rôle. The possibility that these organisms may be foodrobbers has always existed and has not been disproved.

Experimental.

Part I.

(1) Study of fluctuations in infusorial populations with changes in the diet of sheep.

(*a*) Stable fed sheep.
(*b*) Veld grazing sheep.

(2) Comparison of the infusorial populations in veld grazing sheep and wild antelopes in their natural state.

Part II.

(1) Study of the rate of digestion of maize starch within an infusorium from material *in vivo*.

(2) To determine whether infusoria accelerate or assist in the digestion of starch within the rumen.

(3) To determine whether ruminal infusoria accellerate or assist in the digestion of cellulose.

PART I.

(1) (*a*) Stable Fed Sheep.

Technique.

All sheep were dosed with 2·5 litres of tapwater through the fistula daily at 2 p.m. so as to keep the intake of water constant. Material for infusorial

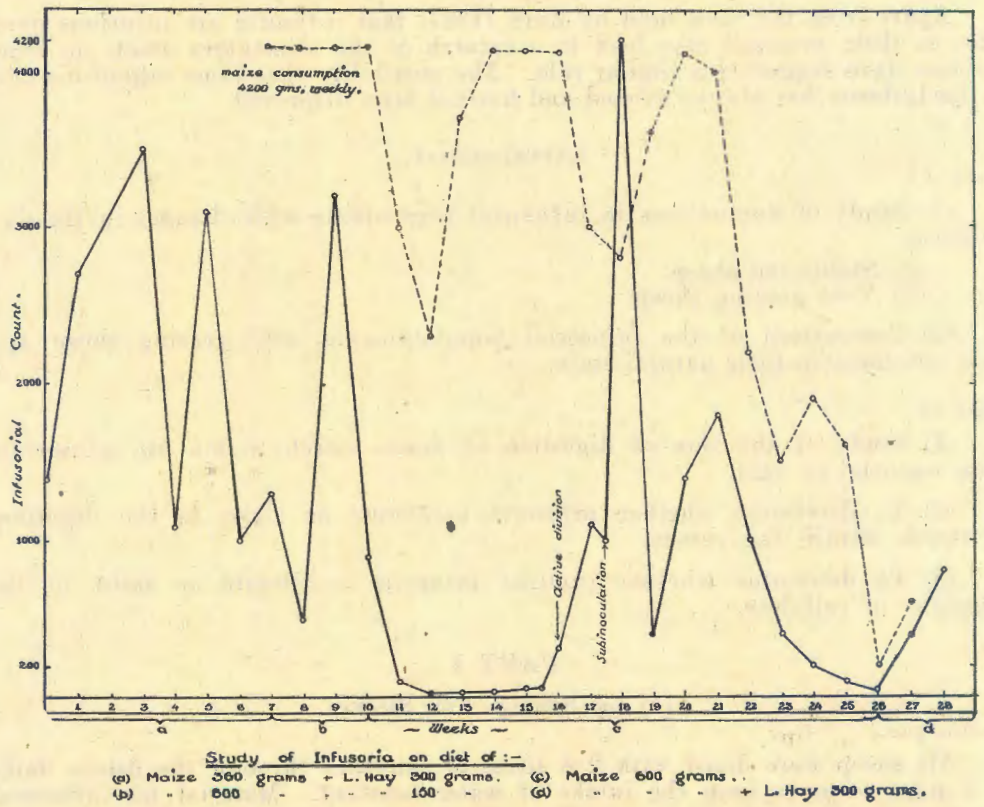
counts was always withdrawn at 9 a.m. After shaking vigorously 1 c.c. was sucked up into a wide mouthed pipette. This was added to 7 or 8 c.c. of corrosive-sublimate-alcohol fixative, and after washing with alcohol-iodine and 70 per cent. alcohol, it was stained with borax-carmine. The stained material was suspended in 3 c.c. oil of cloves in which it can be kept for a long time. After diluting to $^1/_{10}$ or $^1/_{100}$ in oil of cloves a drop of known volume from a capillary pipette was placed on a glass slide and covered with a coverslip. The total number of infusoria per drop was counted, from which the number per cubic millimetre ingesta was then calculated. Duplicate counts were made.

Experiment 1.—(*a*) *Maize and lucerne ration.*
(*b*) *Maize ration.*

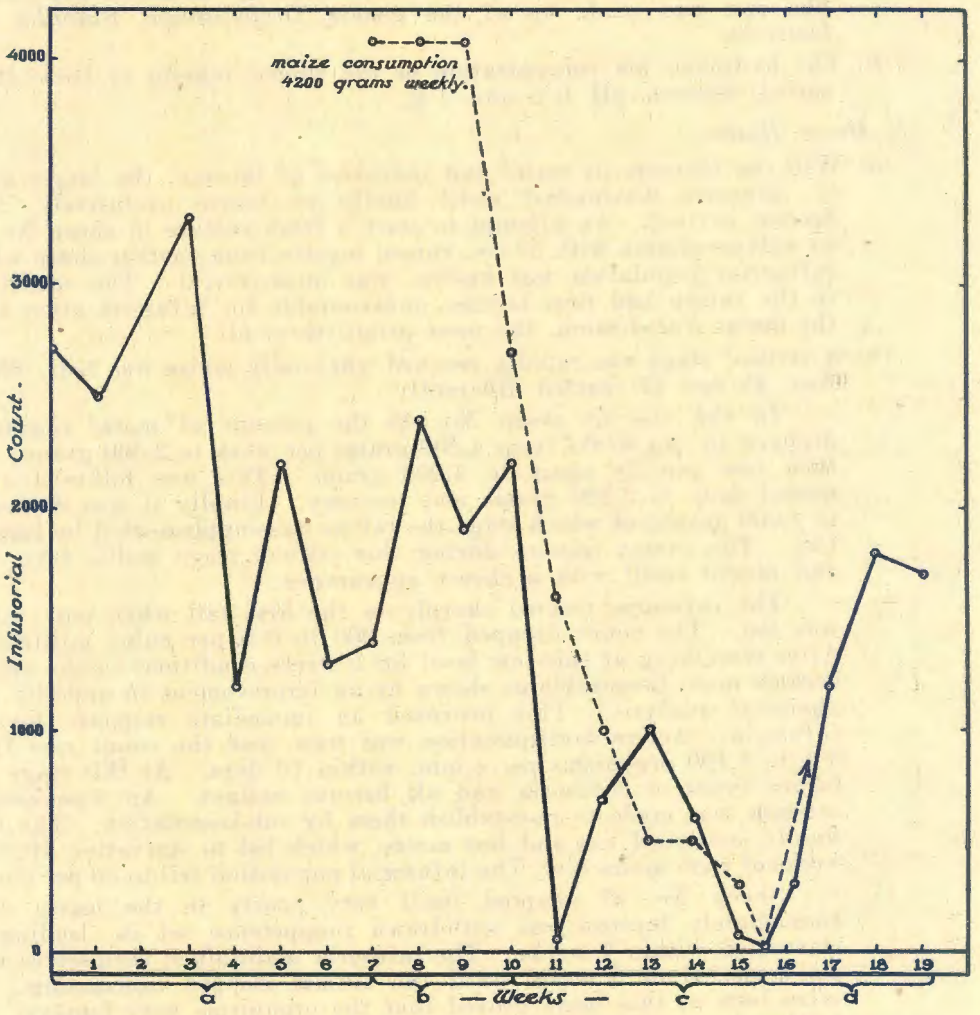
Sheep No. 45 and No. 37 on a mixed ration of 360 grams of crushed yellow maize plus 300 grams of lucerne hay were used in an experiment to follow up the influence of a change from the above ration to a pure maize ration containing approximately 75 per cent. carbohydrate, 10 per cent. protein and 2 per cent. fibre.

Infusorial counts were made weekly, and when necessary, also at shorter intervals. Differential counts were made to correlate adaptive changes in these organisms with changes in the diet. A record of the food consumption of the animals was kept, and is reflected in graphs 1 and 2 opposite the infusorial counts.

Graph I: Sheep 45.



Graph II : Sheep 37.



Study of Infusoria on diet of :-

(a) 360 grams y. maize + 300 grams Lucerne Hay.
(b) 600 " " " 150 " " " "
(c) " " yellow maize.
(d) " " " " + 300 grams Lucerne Hay.

Discussion and Conclusions.

(1) *Maize and Lucerne Ration.*

(*a*) Infusorial population fluctuated between 1,000 and 3,500 per cubic millimetre in sheep No. 45, and between 1,200 and 3,300 in sheep No. 37.

The differential counts disclosed that in both sheep the genus *Entodinium* comprised 95 per cent. of the total infusorial population. The rest was made up of the genera *Diplodinium*, *Bütchlia* and *Isotricha*.

(*b*) The hydrogen ion concentration of the rumen ingesta of these sheep varied between pH 6·6 and 7·2.

(2) *Maize Ration.*

(*a*) With an increase in maize and reduction of lucerne, the larger types of infusoria diminished until finally on maize exclusively, they became extinct. An attempt to start a fresh culture in sheep No. 45 by subinoculation with 50 c.c. rumen ingesta from another sheep whose infusorial population was known, was unsuccessful. The conditions in the rumen had thus become unfavourable for infusoria other than the genus *Entodinium*, the most primitive of all.

(*b*) A critical stage was rapidly reached when only maize was fed. Sheep Nos. 45 and 37 reacted differently.

In the case of sheep No. 45 the amount of maize consumed dropped in two weeks from 4,200 grams per week to 2,300 grams, and then rose rapidly again to 4,200 grams. This was followed by a second drop to 2,800 grams and recovery. Finally it was depressed to 2,000 grams, at which stage the ration was supplemented by lurecne hay. The rumen ingesta during this critical stage had a very sour and rancid smell with a cheesy appearance.

The infusoria reacted sharply to the first fall when only maize was fed. The count dropped from 900 to 0·5 per cubic millimetre. After remaining at this low level for 5 weeks conditions in the rumen became more favourable as shown by an improvement in appetite and chemical analysis. This provoked an immediate response by the infusoria. Active multiplication was seen, and the count rose from 0·5 to 1,100 organisms per c.mm. within 10 days. At this stage the larger types of infusoria had all become extinct. An unsuccessful attempt was made to re-establish them by sub-inoculation. The host finally consumed less and less maize, which led to starvation after 16 weeks of pure maize diet. The infusorial population fell to 30 per c.mm.

Sheep No. 37 adapted itself very poorly to the maize diet. Immediately lucerne was withdrawn inappetence set in, leading to starvation within 7 weeks. The infusoria maintained themselves well for a period of 5 weeks until the animal stopped ruminating. *In vitro* tests at this stage proved that the organisms were hungry, due to the absence of macerated starch particles. The symbiotic harmony between host and infusorium was thus disturbed by failure to ruminate. Supplementation with lucerne hay also induced rapid multiplication.

(*c*) Of the *Entodinium* species *E. nanellum* was the most resistant, and thrived better under conditions adverse to other species. A small number of *E. furca*, *E. simplex*, *E. elongatum* and *E. dubardi* survived as seen in Table 1 below, showing average percentage of *Entodinium* species per cubic millimetre.

(*d*) Chemical analysis of the rumen ingesta is reflected in Table 2. After acidifying, distillation was carried out according to Wiegner's process as reported by Smith (1938). Material was withdrawn through the fistula daily at 9 a.m. before feeding.

TABLE 1.

Sheep.	Period.	*E. nanellum.*	*E. furca.*	*E. simplex.*	*E. elongatum.*	*E. dubardi.*
45	Before maize.....	32	21·5	5·5	26·5	10
	After maize.....	66	12	5	11	6
37	Before maize.....	42	17	8·5	21·5	6
	After maize.....	62	9	7	17	5

It is evident from the table that in sheep No. 45 acetic and butyric acid rose very high at the time when the infusorial population diminished significantly. On the other hand the acetic and butyric acid values kept within normal limits (see sheep 42, 43 and 35) in the case of sheep No. 37 with a pH of 5·4. Under these conditions the infusoria thrived until starved. Excessive amounts of acetic and butyric acid in the rumen are therefore harmful to the infusoria. *In vitro* tests confirmed this. The exceptionally large quantity of maize consumed by sheep No. 45 was the cause of the acidity of its rumen.

Experiment No. 2.—Effect of Wheat Straw Diet on Infusoria.

Three sheep on a diet of 300 grams wheat straw and 360 grams of crushed yellow maize were used for infusorial counts. The amount of maize was first reduced to 100 grams and then omitted with a corresponding increase in wheat straw to 600 grams. Counts were made every second day for 28 days. At this stage the effect on the infusoria of a supplementation of 100 grams yellow mealie meal, introduced directly through the fistula, was tested for a period of eight days. Mealiemeal was then substituted by 100 grams of maize starch to eliminate the protein contained in the mealiemeal. Differential counts were made as in Experiment No. 1. See Graph III and Table 3 below.

TABLE 3.

Period.	Sheep No. No.	*E. nanellum.*	*E. furca.*	*E. simplex.*	*E. elongatum.*	Unidentified *Entodinium* Species.	*Diplodinium* Species.
Wheat straw + maize	37	27	48	4	3	4	1
	40	49	40	3	1·5	6	·5
	43	21·5	58·5	5·5	—	13	·5
Wheat straw only	37	33	12	38	7	6	4
	40	21	18	40	12	5	4
	43	36	20	30	12	12	3·5

Discussion and Conclusion.

(*a*) On a wheat straw and maize diet the infusorial population fluctuated between 700 and 900. The genus *Entodinium* comprised 99 per cent. of the total. Upon reduction and later omission of maize, there was a significant fall in numbers to an average of 50 organisms per cubic mm. as a result of starvation.

TABLE 2.

Distillation of Rumen Ingesta.

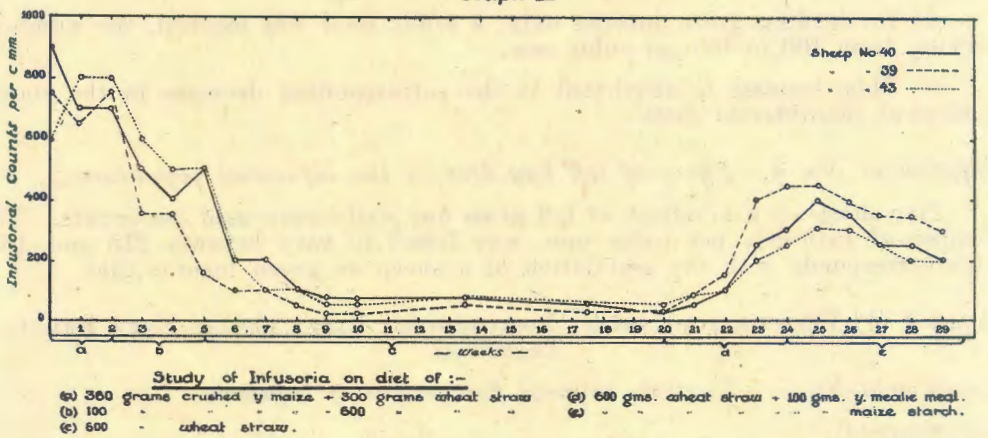
Sheep No.	Date.	Diet.	Acetic Acid.*	Butyric Acid.	Non-Vol. Acids.	Total Organic Acids.	Total Vol. Acids.	pH. of Rumen Ingesta.	Remarks.
42	23. 9.38	300 grams lucerne hay + 360 grams yellow crushed maize	17·4	13·32	4·62	35·34	30·72	6·8	—
43	10.11.38	300 grams lucerne hay + 360 grams yellow crushed maize	17·52	6·44	7·70	31·66	23·96	6·8	—
35	12.11.38	Green lucerne.....	13·42	4·76	1·90	20·08	18·18	6·9	—
32	17.11.38	Green lucerne.....	12·05	6·46	5·50	24·01	18·51	7·0	—
45	30. 9.38	600 grams yellow crushed maize	16·5	9·61	18·20	44·31	26·11	5·5	—
	5.10.38		7·96	3·78	8·00	19·74	11·74	5·4	—
	13.10.38		26·90	4·68	5·60	37·18	21·58	5·4	—
	14.10.38		21·90	2·52	5·72	30·12	24·42	5·4	—
	18.10.38		26·70	10·85	5·74	43·29	37·55	5·2	—
	19.10.38		27·80	9·41	15·70	52·91	37·21	5·9	—
	9.11.38		21·00	4·55	2·50	28·05	25·55	5·6	—
37	29. 9.38	600 grams yellow crushed	15·60	4·58	6·50	26·68	20·18	5·4	—
	1.10.38		18·50	8·68	14·65	41·83	27·18	5·6	—
	6.10.38		14·05	4·45	25·60	44·10	18·50	5·4	—
	13.10.38	600 grams yellow crushed maize	9·90	3·83	7·00	20·73	13·73	5·3	—
	14.10.38		9·24	13·82	21·50	34·56	13·06	5·6	—
	18.10.38		7·64	3·28	8·00	18·92	10·92	5·4	—
	19.10.38		14·40	3·88	14·00	32·28	18·28	5·4	—
	6.11.38	Starvation.....	1·62	0·34	1·50	2·46	1·96	5·4	—
40	19.11.38	Straw.....	14·51	3·91	5·70	24·12	18·42	—	Both Nos. 40 and 39 ate poorly on 22.11.38 and 24.11.38.
	24.11.38	Straw, half-ration.....	14·51	3·91	5·70	24·12	18·42	—	
39	15.11.38	Straw.....	12·40	4·82	4·98	22·20	17·22	—	—
	22.11.38	Straw, half-ration.....	4·24	1·16	5·00	10·40	5·40	—	—

* All acid values expressed as c.c. $\frac{N}{10}$ — NaOH.

On the addition of maize again, the infusoria doubled their numbers within 24 hours, and after a week the count had risen to 500 per c.mm. On replacing the mealiemeal by starch, multiplication was retarded probably to protein deficiency.

(*b*) Of the entodinia *Entodinium simplex* adapted itself best to the wheat straw ration. It increased from 4 per cent. to 35 per cent., whereas *E. furca* diminished from 50 per cent. to 18 per cent. The genus *Diplodinium* increased from ·5 to 4 per cent.

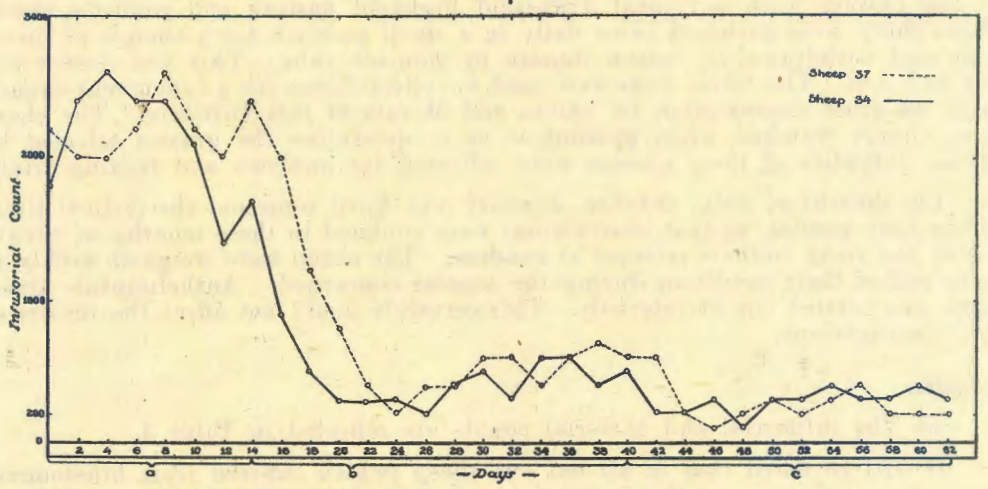
Graph III



Study of Infusoria on diet of :-

(a) 360 grams crushed y maize + 300 grams wheat straw
(b) 100 - - - - 600 - - -
(c) 600 - wheat straw.
(d) 600 gms. wheat straw + 100 gms. y. mealie meal.
(e) - - - - - - maize starch.

Graph IV



Study of Infusoria on diet of :-

(a) 360 grams crushed maize + 300 grams L.Hay.
(b) 1 K.gram dry Lucerne Hay.
(c) 3 K.grams green Lucerne Hay.

Experiment No. 3.—*Comparison of infusorial counts in sheep on a diet of:*

(1) 360 grams of maize and 300 grams dry lucerne hay.
(2) 1 Kgm. of dry lucerne hay.
(3) 3 Kgm. of green lucerne.

Infusorial counts were made every second day for periods of three weeks successively on sheep which were on the above diets. The results are recorded graphically in Graph IV.

Conclusions.

(*a*) On maize + lucerne hay the population fluctuated between 1,400 and 2,600 per cubic mm. With omission of maize the number of infusoria decreased rapidly and established a new level fluctuating between 200 and 700 per cubic mm.

(*b*) On feeding green lucerne only, a lower level was reached, the numbers varying from 100 to 400 per cubic mm.

(*c*) This decrease is attributed to the corresponding decrease in the starch content of the different diets.

Experiment No. 4.—Effect of teff hay diet on the infusorial population.

Five sheep on a kilogram of teff grass hay daily were used for counts. The number of infusoria per cubic mm. was found to vary between 215 and 485. This corresponds with the population of a sheep on green lucerne diet.

PART I (*b*) VELD GRAZING SHEEP, NOOITGEDACHT EXPERIMENTAL FARM ERMELO, TRANSVAAL.

Experiment No. 5.—To study seasonal fluctuations of Infusoria.

Experimental:

A group of 40 healthy merino wethers were allowed to graze freely in a camp of 100 morgen with a typical Transvaal highveld pasture and running water. These sheep were gathered twice daily in a small paddock for a change of faeces bags and withdrawal of rumen ingesta by stomach tube. This was done every day at 7 a.m. The faeces bags were used to collect faeces for a concurrent experiment on grass consumption by Smuts and Marais of this Institute. The sheep were closely watched when grazing so as to determine the grasses selected by them. Supplies of these grasses were collected for analyses and feeding trials.

The months of July, October, January and April represent the critical times of the four seasons, so that observations were confined to these months on twenty out of the forty wethers selected at random. The sheep were weighed weekly so as to reflect their condition during the seasons concerned. Anthelminthic treatment was carried out at intervals. This certainly could not affect the results of the investigations.

Results.

(*a*) The infusorial and bacterial counts are reflected in Table 4.

It will be noted that in all but one sheep (which suffered from bluetongue) there is an increase in the infusorial and bacterial populations from July to January, and a decrease from January to April, the April counts corresponding to some extent with those of October.

There was an average of 100 infusoria per cubic millimetre in July, 277 in October, 455 in January and 278 in April. The bacteria averaged 1067×10^6 per cubic centimetre in July, 1889×10^6 in October, 1944×10^6 in January and 1756×10^6 in April respectively.

TABLE 4.

Seasonal fluctuations in infusoria of veld grazing sheep, Nooitgedacht, Ermelo (organisms per cubic millimetre).

Sheep No.	July.	October.	January.	April.
52100	80·8	350·0	359·6	351·0
51840	57·8	189·0	497·8	224·0
52079	50·3	150·1	455·4	333·3
52082	42·3	250·9	426·1	298·7
51652	170·1	240·5	315·4	228·0
51904	20·2	175·5	379·2	256·6
51765	144·2	162·2	451·3	372·4
51741	117·0	224·0	162·4	125·4 (+bluetongue)
51833	132·1	152·7	780·2	376·2
51738	119·8	270·5	345·8	171·0
51885	77·6	369·4	530·7	294·4
52030	138·6	420·6	451·8	301·8
51812	96·4	319·6	485·0	281·0
51693	150·8	427·7	510·6	326·8
51757	59·6	315·0	420·7	317·8
52010	29·6	315·0	570·0	209·0
52025	30·5	425·6	470·9	260·5
51694	128·9	330·4	451·0	279·6
51689	183·7	166·8	430·0	288·8
51738	160·1	288·0	560·5	171·0
AVERAGE PER C.MM.	100	277	455	278

In July, 20·6 per cent. of the infusoria consisted of the larger types, i.e. excluding the genus Entodinium; in January this figure had fallen to 15·7 per cent. This difference is due to the fact that the large types thrive better than the Entodiniums on lignified diets. The Entodinium species flourish when diets are rich in starch and nitrogen.

(*b*) Comparison of Counts and Feeding Conditions.

In Table 5 is given a complete summary of the nutritional quality of the grazing during the different seasons of the year as well as the average infusorial and bacterial counts of sheep subsisting on it. An interesting and significant feature in this respect is the marked and prominent fluctuation in nutritional conditions during the year. From October to March there is a super-abundance of a fairly good quality grazing as a result of which both the maintenance and the growth requirements can be met. However, this condition is completely reversed during April to September. In this interval grazing is extremely poor. The protein drops from an average of 9 per cent. to approximately 3 per cent. (Smuts and Marais, 1940; Smuts and Marais, 1940). Together with this rapid decline in protein there is a tremendous increase in fibre. These factors are closely linked with the stage of maturity of the grazing. The grazing conditions under which this experiment was carried out are therefore very variable in nature. Thus, in certain seasons there was an abundance of nutrients available while a deficiency existed during the rest of the year. Such fluctuating nutritional conditions, as can be readily appreciated, must tax the digestive system,

the process of utilization, as well as the general health and vitality of the animal in a severe manner. Physiologically one must assume that the normal digestive powers and reactions together with the intricate functions of utilization of nutrients cannot be best accomplished below a certain level of nutrition. Consequently it appears that while the processes of assimilation and utilization of feed form an inseparable physiological unit, the reactions experienced by one function will automatically be reflected by the other.

TABLE 5.

	July.	October.	January.	April.
Average weight of sheep.....	28 Kgm.	33	40	39
Dry grass consumed.....	649 gm.	785	907	861
Per cent. nitrogen.....	0·49	1·44	1·17	0·73
Biological value.....	83	62	74	82
Digestibility of nitrogen.....	2 %	60 %	51 %	12 %
Digestibility of dry matter.....	43	58	60	46
Nitrogen balance.....	− 2·40	0·46	0·53	− 1·40
Number of infusoria per cubic mm.....	98	277	455	273
Number of bacteria per cubic cm.....	1037×10^6	1889×10^6	1944×10^6	1756×10^6

In other words there should exist a close relationship between quality and utilizability of feed and the physiological factors concerned in the digestion of such feed; that such a relationship actually appears to exist is evident from a comparison between the protein content of the grazing, the digestibility and the infusorial and bacterial populations. Whether such a relationship is connected primarily with the protein in the grazing or with absence or presence of other nutrients or finally with the vitality of the animal is difficult to assess at present. It is nevertheless remarkable that a low protein content or a deficient nutritional state, which invariably affects the vitality as well as the health of the animal, markedly reduces the infusorial and bacterial populations. From these observations it would appear that the multiplication and normal influence of infusoria and bacteria in digestion depends largely on a suitable substratum in the rumen and reticulum. Such a suitable substratum, the composition of which is as yet not fully appreciated, is dependent on the nutritional condition of the animal. That protein must play an important part as a necessary component of such a substratum is obvious from Table 5 where the infusoria and bacteria decrease almost proportionately with the decrease in protein content.

This fact is furthermore substantiated by the observation previously indicated in Graph III that the infusorial population decreases on a nitrogen low diet composed of maize starch and a roughage.

II. COMPARISON OF THE INFUSORIAL POPULATIONS OF VELD GRAZING SHEEP AND ANTELOPES IN THEIR NATURAL STATE.

Buisson (1923) published an account of the various species of ciliates found in the African rhinoceros and elephant. In 1924 he described the ciliates present in African antelopes from the Belgian Congo. Two species of the genus *Entodinium* and two of genus *Diplodinium* described by Buisson in these antelopes have been found by Fantham (1925) and Schuurman (1926) to occur in South African sheep and cattle.

Dogiel (1925) obtained his material in 1914 from Lake Naivasha and Kilimanjaro in East Africa. He examined material from six different species, which are also indigenous to South Africa. This led to the description of a number of new species of ciliates. Most of the species described by Dogiel, excepting the species *Diplodinium costatum* and the genus *Opistotrichum* have been observed by Fantham in their studies on sheep and cattle.

The genus *Ophryoscolex* seems to have been well represented in South African sheep and cattle as well as in East African antelopes. Schuurman expressed the opinion that our cattle and sheep became infected from antelopes on the same veld. Apart from the above systematic work on South African sheep and cattle and on East African antelopes, no work has been done to ascertain firstly, the relationship between the infusorial populations of antelopes on different natural diets and, secondly, between antelopes and domesticated animals on comparable diets.

To gain information on these points one of the writers (J. G. v. d. W.) made use of an opportunity to accompany Dr. A. D. Thomas of this Institute on a Zoological Survey collection tour into the Transvaal Lowveld. During this expedition ten species of antelopes were shot. Ruminal material was collected immediately afterwards and treated as outlined before. Duplicates from some species were collected at different localities.

Table 6 shows the species, its natural diet, the differential count between small and large types of infusoria and the total infusoria per cubic millimetre.

Table 7 shows the dominant ciliate(s) in the various antelopes.

Discussion and Conclusions.

(*a*) Antelopes may be divided roughly into two classes according to their natural diet. Under normal conditions the Klipspringer, Duiker, Impala and Kudu feed almost exclusively on legumes, leaves of certain trees and shrubs, and berries. They are also very fond of young and tender grass and green cereals e.g. oats and wheat. The Duiker also digs up roots and tubers. On the other hand the Steenbuck, Reedbuck, Waterbuck, Sassaby, Sable Antelope and Blue Wildebeest feed almost exclusively on grass and reeds. In times of scarcity they also feed on leaves and legumes. The Steenbuck is a very delicate feeder and selects only the finest and tenderest grasses.

The difference between the two types of diet is significant; camel thorn pods and Mopani leaves contain 12·5 per cent. and 12·1 per cent. protein respectively. Other legumes and leaves were not analysed but could be considered to correspond closely to the above figures. Berries, roots and tubers contain a high percentage of carbohydrate. The protein and carbohydrate contents of this diet are therefore considerably higher than a diet of grass which contains approximately 4 per cent protein and very little starch in the lowveld during the month of July. Antelopes feeding on a diet rich in starch and protein have a very frothy ruminal ingesta with large amounts of gas escaping, whereas those grazing on grass do not develop such an active ruminal fermentation.

Table 6 shows a significant difference in the total infusorial counts as well as in the proportions of the various types of infusoria present. This could be very closely correlated with the diet. The group with the richer protein diet harbours more than five times the number of infusoria than the group on the low protein diet does. The former group (browsers) shows a ratio of 1:2·8 between large and small infusoria (genus *Entodinium*), whereas in the later group

(grazers) the ratio is 2:1. In antelopes, as in sheep, the larger types of infusoria thrive better than the smaller types on diets containing a high proportion of cellulose, probably because they are able to ingest the larger cellulose particles better. In diets rich in protein and carbohydrates, the genus *Entodinium* can maintain itself better and thus proportionately outnumbers the larger types.

In sheep grazing freely on the veld in the month of July when the protein was at its lowest for that year (2·9—3 per cent.), the larger infusoria comprised 20·6 per cent. of the total population. In January when the protein rose to its highest level (9 per cent) there was a drop to 15·7 per cent. in the number of large infusoria.

The average number of infusoria per antelope of the grazer group in July was 313 per cubic millimetre. This is comparable to the average of 278 and 277 in sheep during April and October when the condition of highveld pastures approximates that of some midwinter lowveld pastures.

TABLE 6.

No.	Species.	Natural Diet.	Differential Count.		Total Infusoria per c.mm.
			G. entodinium.	*G. diplodinium.*	
1	Transvaal Klipspringer: *Oreotragus oreotragus*, Roberts....	Browser	122	94	1161
2	Transvaal Duiker: *Sylvicapra grimmi*, Roberts.....	Browser	275	5	1260
3	*Sylvicapra grimmi*, Roberts.....	Browser	502	170	3024
4	"Rooibok", Impala, Typical Impala: *Aepyceros melampus*, Lcht.....	Browser	143	82	1012
5	*Aepyceros melampus*, Lcht.....	Browser	1347 (E.R.)	511	8271
6	*Aepyceros melampus*, Lcht.....	Browser	203	86	1296
7	Zambesi Kudu: *Strepsiceros strepsiceros*, Lorenz...	Browser	108	59	751
8	Lowveld Steenbuck: *Raphiceros rufescens*, Thos. and Schw.....	Grazer	100	63	376
9	Reedbuck—Rietbok: *Redunca arundinum*, Bodd.....	Grazer	7	59	297
10	Waterbuck—Waterbok: *Cobus ellipisiprymnus*, Ogilby....	Grazer	32	102	303
11	Sassaby, Basterhartbees: *Damaliscus lunatus*, Burch.....	Grazer	13	62	337
12	Swartwitpens, Transvaal Sable Antelope: *Ozanna nigra*, Harris.....	Grazer	14	27	184
13	*Ozanna nigra*, Harris.....	Grazer	27	42	310
14	Blue Wildebeest—Blou wildebees: *Gorgon taurinus*, Burch.....	Grazer	18	32	224
15	*Gorgon taurinus*, Burch.....	Grazer	39	66	472

(*b*) The dominant species of infusoria in antelopes of the same species do not differ. In the different Blue Wildebeest the dominants were *Eudiplodinium neglectum Dog.* (1925) *forma gigantium Dog.* (1925) and *Entodinium simplex Dog.* 1925. In the Sable Antelope the dominants were *Eudiplodinium maggii Fior.* (1889) and *Entodinium caudatum Stein* (1859). In Impala *Epidinium ecaudatum Fior.* (1889) and *E. simplex Dog.* (1925) dominated. In the Duiker the dominants were an undescribed *Eudiplodinium* species and *Entodinium nanellum.*

TABLE 7.

Antelope.	Dominant Ciliate(s).	
	Large Types.	Small Type (Entod.).
1. Blue Wildebeest.....	(i) *Eudiplodinium neglectum forma giganteum*, Dog. (1925) (ii) *Anoplodinium bubalidis forma consors*, Dog. (1925)	*Entodinium simplex*, Dog. (1925).
2. Blue Wildebeest.....	(i) *Eudiplodinium neglectum forma giganteum*, Dog. (1925) (ii) *Anoplodinium bubalidis forma bubalidis*, Dog. (1925)	*Entodinium simplex*, Dog. (1925).
3. Sable Antelope.....	*Eudiplodinium maggii*, Fior. (1889)	*Entodinium caudatum*, Stein (1859).
4. Sable Antelope.....	*Eudiplodinium maggii*, Fior. (1889)	*Entodinium caudatum*, Stein (1859).
5. Sassaby.....	*Eudiplodimium neglectum forma giganteum*, Dog. (1925)	*Entodinium nanellum.*
6. Waterbuck.....	(i) *Eudiplodinium maggii* (ii) *Ostracodinium gracili forma gracili*, Dog. (1925)	*Entodinium dubardi gracilicaudatum*, Buisson (1923).
7. Reedbuck.....	(i) *Ostracodinium gracili forma gracili*, Dog. (1925) (ii) *Anaplodinium costatum forma minor*, Dog. (1925) (iii) *Eudiplodinium maggii*	*Entodinium caudatum*, Stein (1859).
8. Steenbuck.....	(i) *Epidinium caudatum forma*, Fior. 1889); *quadricaudatum*, Sharp (1914) (ii) *Anaplodinium costatum major*, Dog. (1925)	*Entodinium triacum*, Buis. (1923). *forma triacum*, Dog.
9. Impala.....	*Epidinium ecaudatum*, Fior. (1889); *forma caudatum*, Fior. (1889)	*Entodinium simplex*, Dog. (1925).
10. Impala.....	*Epidinium ecaudatum*, Fior. (1889); *forma caudatum*, Fior. (1889)	*Entodinium simplex*, Dog. (1925).
11. Impala (E.R.).....	*Epidinium ecaudatum*, Fior. (1889); *forma caudatum*, Fior. (1889)	*Entodinium simplex*, Dog. (1925).
12. Kudu.....	(i) *Eudiplodinium neglectum*, Dog. (1925); *forma impalae*, Dog. (1925) (ii) *Epidinium ecaudatum*, Fior. (1889), form undescribed	*Entodinium simplex*, Dog. (1925).
13. Duiker.....	*Eudiplodinium* species undescribed	*Entodinium nanellum.*
14. Duiker.....	*Eudiplodinium* species undescribed	*Entodinium nanellum.*
15. Klipspringer.....	*Eudiplodinium* species undescribrd, different from Nos. 13 and 14	*Entodinium triacum*, Buis. (1923); *forma triacum*, Dog.

(*c*) Different species of antelopes grazing on the same veld do not harbour the same dominant infusoria excepting in the case of the Blue Wildebeest and the Sassaby. The dominant organisms in these did not appear in the Sable

Antelope, Waterbuck or Reedbuck found in the same veld, nor in any of the browsers of the same locality. The dominant organism in the Impala namely *Ent. simplex Dog.* (1925) did appear in the Wildebeest as the dominant of the smaller type of infusoria but only in very small numbers.

(*d*) Impala, from an area where owing to lack of grazing, more browsing is done, showed up to eight times more infusoria than Impala in areas where young green grass and young shoots are abundant.

(*e*) The genus *Ophryoscolex* was not seen in sheep or antelopes.

(*f*) The sub-genera *Eudiplodinium neglectum Dog.* (1925), *Ostracodinium* and *Opistotrichum* were not seen in any of the sheep examined.

(*g*) The genus *Epidinium* although present both in antelopes and sheep, occurs more frequently in the former.

The genus *Diplodinium* on the other hand occurs with greater regularity in sheep. The genus *Entodinium* is commonly seen in both and is invariably the dominant organism in animals on diets rich in protein and carbohydrates.

(*h*) Several undescribed species were seen in the material examined. These will be described in due course.

PART II.

1. Study of the rate of Digestion of Maize Starch within an Infusorium from material in vivo.

As the results of the experiments described under Part I (i) indicated a probable significant rôle of infusoria in so far as the digestion of starch is concerned, it was decided to investigate this possibility by studying the actual digestion of starch, firstly, within the organism itself and secondly, within the rumen of the sheep in the ordinary process of digestion.

Experiment No. 1.—*Digestion of starch within an infusoria from material in vivo, i.e. material withdrawn from the rumen of sheep through the fistula.*

For this experiment sheep were fed wheat hay free of starch granules. This approximates starvation of the infusoria and enables one to follow up closely the intake and digestion of starch granules administered into the rumen.

Sheep Nos. 39, 40, 43 and 45 were used. Their infusoria were examined daily by staining fresh drops of rumen ingesta on a slide with Grams iodine to differentiate starch and glycogen. As soon as the infusoria were found to contain no more traces of starch or glycogen, 2 grams of finely sifted yellow maize meal were given through the fistula tube of each sheep at the desired time. One minute afterwards material was withdrawn and immediately examined microscopically. Further samples were collected at thirty minute intervals, and later at hourly, and longer intervals, and examined without delay. In this way a complete picture was obtained of the process of digestion of starch. The amount of starch dosed was small and quickly ingested by the infusoria so that uningested starch grains were only occasionally found after an hour or more.

It could therefore be safely assumed that the infusoria studied by periodic withdrawal from the rumen had ingested the starch at or soon after the time of dosing. With doses of 10 and 20 grams of mealiemeal, free, partially digested starch granules could be found in the rumen 18 hours later.

As the results of the periodic examinations at different times and with different sheep were all in very close agreement, only one such report will be given here. The average rate of digestion is illustrated better by the photomicrographs submitted in Plates I and II.

Rate of digestion of 2 grams of fine yellow mealiemeal.

9.10 a.m. Ruminal ingesta withdrawn and examined by staining with Gram's iodine. Both *Entodinium* species and *Diplodinium* species appeared hungry. Some *Diplodinium* contained cellulose material.

9.15 a.m. Dosed 2 grams yellow mealiemeal.

9.16 a.m. (1 minute) Material withdrawn and examined. Most organisms had already ingested starch grains, particularly the *Entodinium*, some containing up to 7 grains.

9.45 a.m. (30 minutes). Practically every organism contained one or more starch grains, some being completely engorged and distended. No evidence of glycogen.

10.15 a.m. (1 hour). Brownish-red granules are appearing in most infusoria.

10.45 a.m. (1½ hours). Many more brownish-red granules are present now.

11.45 a.m. (2½ hours). Organisms previously engorged with starch grains are now packed with glycogen-like granules.

12.45 (3½ hours). Starch grains are now becoming obscured by brown granules.

2.45 (5½ hours). In engorged organisms no change observed. Those with one or two grains only, show signs of disintegration of the starch comparable to karyolysis, with many brown granules around the disintegrating grain.

4.45 p.m. (7½ hours). The processes are much more advanced.

9.45 p.m. (12½ hours). Disintegration of the starch grain is now taking place as in simple diastatic digestion of starch. There is complete loss of its original globular form and staining affinity. The deep violet changes to a pale blue and then to bluish-brown. Masses of brown granules are now present in ecto- and endoplasm giving the organism a deep dark-brown granular appearance (see Plate No. 3).

9 a.m. On following day (24 hours). Brown granules are markedly reduced in the organisms. Colourless, transparent granules are now seen in increasing numbers in the regions previously occupied by brown granules.

3 p.m. (30 hours). Still fewer brown granules.

9 p.m. (36 hours). Glycogen granules clearing up rapidly.

9 a.m. next day (48 hours). All brown granules have disappeared and numerous colourless transparent granules have taken their place.

Discussion and Conclusions.

(1) Within 48 hours after ingestion the maize starch is completely digested and utilised within an infusorium. This confirms the results obtained *in vitro* by Trier (1936). Mangold (1929) quotes Trier extensively to explain the ways in

which starch granules are ingested by infusoria. Trier found that as a result of intracellular digestion within the organism glycogen granules appear in their ectoplasm. He thus assumed that there was an intracellular synthesis of glycogen by the infusorium which is utilised by the organism itself. The infusoria is said to perform this breakdown of starch by an endogenous diastatic enzyme.

We have, however, found that free starch grains within the rumen, when not ingested by infusoria, are digested at the same rate by being directly attacked by bacteria and fungi. It should be noted that the saliva of the ruminant does not contain a diastic enzyme as suggested by Westphal (1934).

Furthermore, by adding 1 per cent. glucose or maltose to ruminal juice containing starved infusoria and incubating at 39° C. for one or two hours with periodic shaking, it was found that infusoria do take in fluid material from their surrounding medium, as in less than an hour after adding the sugar, glycogen granules began to form within the organism. After three or four hours the organisms were packed with brown granules similar to those seen after a heavy starch meal.

By staining fresh preparations of rumen ingesta intra-vitally with Janus Green we could establish beyond doubt, that the socalled glycogen granules as well as the colourless transparent granules were actually bacteria situated within the plasma of the infusoria. Most of these bacteria show typical Brownian movement and change their position within the ectoplasmic cavity. Large numbers of bacteria mixed with debris are present in the foodsac or body cavity and are rotated by the energetic movements of the membranelles. With ingestion of starches or sugars by the infusorium or food material containing starch or sugar, these substances are digested by enzymes secreted by the bacteria present there. Such bacteria as are able to synthesize glycogen within their own bodies utilise the products of digestion of the food material present and react to glycogen stains. The infusorium thus has within its body a process of digestion from which it derives definite benefits without any digestive contribution of its own excepting for its capacity as host. Ample proof of the advantages gained by the infusorium is afforded by the fact that rapid multiplication follows whenever the socalled " glycogen granules " appear in some measure after a feed. The fact that uningested starch grains are digested by free ruminal bacteria at the same rate as starch grains within an infusoria, proves that no enzymatic contribution is made by the infusoria itself towards the digestion of starch, and that it is wholly dependent upon ruminal bacteria and bacterial action for its own nourishment and the synthesis of glycogen within its body. It is probably for this reason that Westphal could not keep or promote multiplication of ruminal infusoria in cultures for any length of time without the daily addition of fresh ruminal juice.

The question as to how these bacteria gain entrance from the foodsac to the ectoplasm is still to be investigated. The fermentation products of starches, cellulose and sugars bathe the organisms in the foodsac and probably reach those in the ectoplasm by simple diffusion out of the foodsac.

The synthesis of glycogen by bacteria, once the necessary substrata are available, is a common occurrence in the rumen as numerous bacteria and moulds in the ruminal juice show typical glycogen staining after a meal of glucose, maltose or starch. After a time, if no more of these substances are available, the glycogen containing bacteria and fungi lose their staining affinity, and become colourless, the larger ones becoming transparent, as in the case of those trapped by the infusoria.

2. To Determine whether Infusoria Accelerate the Intraruminal Digestion of Starch.

That infusoria are intimately linked up with the digestion of starch within the rumen is the natural conclusion drawn at first sight considering the large numbers of these organisms consuming a considerable amount of starch when available. However, the results of the previous experiment nullify any significance that infusoria may have been believed to have in starch digestion. The following experiment was planned in order to confirm this. It was decided to compare the rate of disappearance of a given quantity of finely sifted yellow mealie meal dosed into the rumen of a sheep containing its normal infusorial population, with the rate of disappearance from the same animal after sterilizing its ruminal fauna of infusoria.

For this purpose two sheep were selected and placed on a grass hay diet containing no chemically detectable starch. An amount of 20 grams of mealiemeal was then dosed daily through the fistula tube in order to establish an infusorial population of 800-1,000 per cubic millimetre. When this was reached samples of ruminal contents were withdrawn for quantitative chemical determination of starch 10 minutes, 5 hours, 9 hours, 12 and 15 hours after dosing. The extraction procedure described by C. S. Hanes (1936) and the chemical methods of Edwards *et al.* (1938) were followed.

After a series of analyses with satisfactory results the animals were sterilised of their infusoria by dosing each with 2 grams of copper sulphate in 2 per cent. solution on three consecutive days following 24 hours starvation and allowing water *ad libitum*. Microscopic examinations over a period of ten days were all negative. Not only all the infusoria exterminated but also the starch attacking cocci previously present, and of which pure cultures had been obtained. These organisms were cultivated again in the rumen of both sheep by inoculating each of them with fairly heavy cultures from six plates. Mealiemeal (20 grams) was dosed daily as before, to encourage the bacterial culture to develop. When these organisms could be seen attacking starch grains as before, sampling was commenced and continued until conclusive results were obtained. See Table 8.

Discussion and Conclusions.

(1) Owing to the anatomical structure of the forestomachs it is impossible to collect for any period of time, material passing from the rumen and reticulum to the omasum and abomasum through the omasal groove. Hence the quantity and composition of ingesta passing through the omasal groove is unknown. For this reason it was impossible to determine the amount of starch which passed out of the rumen undigested, so that the estimations were confined not to the rate of digestion of starch but to the rate of disappearance from the rumen. This then includes the amount of starch digested in the rumen as well as that passed out of the rumen. Under controlled conditions of feeding and watering the latter could be taken as constant over periods of 12 or 24 hours.

(2) In both sheep 20 grams of yellow mealiemeal had completely disappeared by the 15th hour when the rumen had an infusorial population of 800-1,000 organisms per cubic millimetre. After sterilising the rumen of both sheep from their infusoria and inoculating the rumen with cultures of starch-splitting cocci usually present, but destroyed by $CuSO_4$ dosage, there was no decrease in the rate of disappearance of the same quantity of mealiemeal.

TABLE 8.

Rate of Disappearance of Starch from Rumen.

Date.	Amount of Starch at Different Periods after Dosing.					Remarks.
	10 Minutes	5 Hours.	9 Hours.	12 Hours.	15 Hours.	
I. Sh. 49.						NOTE.—The amount of starch is expressed in grams per 100 grams ingesta.
27.11.39	0·130	—	—	0·018	0·010	An iodophilic coccus was noticed to be present in fairly large numbers and competing with the infusoria in the breakdown of strach.
29.11.39	0·117	—	—	0·054	0·032	
1.12.39	0·156	—	—	0·016	Negative	
15.12.39	0·120	—	—	0·037	0·012	
17.12.39	0·083	—	—	0·033	Nagative	
19.12.39	0·122	—	—	0·026	Negative	
21.12.39	0·148	—	—	0·014	Negative	
	After sterilisation with $CuSO_4$ and inoculation with Iodophilic bacteria.					On 3.12.40 the sheep was inoculated intraruminally with six plates of pure culture of the Iodophilic coccus which was destroyed by $CuSO_4$.
2. 1.40	0·073	—	—	Trace	Negative	
4. 1.40	0·053	—	—	Trace	Negative	
6. 1.40	0·074	—	—	0·014	Negative	
II. Sh. 43.						
23. 2.40	0·083	0·050	0·020	—	Trace	An Iodophilic coccus similar to the type seen in Sheep No. 49 as well as an Iodophilic bacillus were present in fairly large numbers attacking starch grains.
26. 2.40	0·087	0·017	0·008	—	Trace	
28. 2.40	0·069	0·036	0·016	—	Trace	
2. 3.40	0·058	0·022	Trace	—	Negative	
3. 3.40	0·059	0·039	0·025	—	Trace	
	After sterilisation with $CuSO_4$ and inoculation with Iodophilic bacteria.					
13. 3.40	0·076	0·012	Negative	—	Negative	On 10.3.40, sheep was inoculated with six plates of pure culture of Iodophilic organisms as in the case of Sheep No. 49. No culture being available, the bacillus could not be inoculated into the rumen, it having also been destroyed by the $CuSO_4$.
15. 3.40	0·076	0·041	0·011	—	Trace	
17. 3.40	0·055	0·025	Trace	—	Negative	
19. 3.40	0·089	0·054	0·014	—	Trace	
21. 3.40	0·085	0·062	Trace	—	Nagative	

If infusoria did play a rôle in the digestion of starch one would have expected to find significant undigested starch residues after 15 hours owing to the absence of infusoria to digest it. It is, however, clear that the function of these organisms is taken over entirely by the starch-splitting bacteria and moulds of the rumen.

3. TO DETERMINE WHETHER INFUSORIA ASSIST IN THE DIGESTION OF CELLULOSE.

The rate of digestion of crushed lucerne stalks was determined in sheep with and without infusoria. For this purpose silk bags as described by Quin, van der Wath and Myburgh (1938) were used. Known weights of a sample of crushed lucerne stalks were suspended through the fistula tube by means of a

silk thread and exposed to ruminal digestion for periods of 24 and 48 hours. Duplicate bags were suspended each time, one being withdrawn at 24 and the other at 48 hours. The sheep were kept on an adequate dry lucerne hay diet during the experiment. Analyses for average percentage cellulose in residues were made and compared with the percentage cellulose present in the homogeneous stock from which the samples were taken. For cellulose determinations the method described by Compton and Maynard (1938) was followed.

The experiment was done first in two sheep, the paunches of which were sterilised of infusoria with $CuSO_4$ three weeks before. Subsequently these sheep were infected with infusoria and when the fauna was well established the experiment was repeated.

Table 9 shows the results of the analyses.

Discussion and Conclusions.

As seen from the analyses the results tend to show an increase in the amount of cellulose digested within the first 24 hours when infusoria were present in the rumen. It was, however, found in the course of another experiment on the same and other sheep with normal unsterilised fauna, that the rate of cellulose digestion may vary by 5 per cent. in 24 hour periods under controlled conditions of feeding and watering. The results, therefore, do not justify at this conclusion that infusoria have a beneficial effect on cellulose digestion. For statistical purposes the experiment should be extended to include several more animals.

From the table it appears that the rate of cellulose digestion is uneven over a period of 48 hours. An average of 13·2 *per cent.* cellulose was digested within the first 24 hours whereas an average of 15·6 *per cent.* was digested over a period of 48 hours. Thus during the second 24 hour period, 2·4 per cent. only was digested. This decrease in rate of digestion is probably due to the fact that the portion of cellulose more exposed to attack by bacteria and enzymes is broken down first, and that the more incrustated and deeper seated cellulose is only gradually reached by digesting influences. It was clearly demonstrated by Baker and Martin (1937) that in the caecum of the horse and the rabbit cellulose particles are attacked by specific organisms which either adhere to the surface or penetrate into the substance of these particles. We have observed that this is also true for cellulose particles exposed to digestion in the rumen. In addition there is the possibility that ruminal juice may be rich in enzymes secreted by cellulose digesting bacteria and which bathe the cellulose particles.

The mechanisms employed in ruminal digestion of cellulose is therefore on a par with ruminal digestion of starch. The digestion of cellulose within the body of the infusorium is also considered to be primarily due to the enzymes of cellulose digesting bacteria ingested by the infusorium. On this basis it is believed by the authors that the ciliates contribute nothing towards the ruminal digestion of cellulose. This supports Mangold's theory that cellulose breakdown does occur within the infusoria by means of similarly ingested cellulose digesting bacteria.

Summary.

(1) A technique is described for the preservation and counting of ruminal infusoria.

(2) Reactions of specific infusoria as well as total infusorial populations to changes in the diet of stable fed sheep were investigated.

TABLE 9.

Ruminal Digestion of Cellulose.

Sheep No.	Condition of Rumen.	Period of Exposure to Digestion.	Number of Periods Exposed.	Average Per Cent. Total Loss.	Average Per Cent. Loss due to Leaching.	Per Cent. Cellulose in Sample.	Average Per Cent. Cellulose in Residues Calc. on Original Weight of Sample.	Average Per Cent. Cellulose Digested. (*a–b.*)
58.....	Free from infusoria	24 hours	4	41·2	25·5	40·9	30·0	10·9
59.....	Free from infusoria	24 hours	4	41·0	25·5	40·9	28·3	12·6
58.....	Free from infusoria	48 hours	3	46·8	25·5	40·9	26·1	14·8
59.....	Free from infusoria	48 hours	3	49·9	25·5	40·9	23·8	17·1
58.....	Infected with infusoria	24 hours	3	40·3	25·5	49·9	27·0	13·9
59.....	Infected with infusoria	24 hours	3	43·6	25·5	40·9	25·7	15·2
58.....	Infected with infusoria	48 hours	3	43·3	25·5	40·9	26·9	14·0
59.....	Infected with infusoria	48 hours	3	48·5	25·5	40·9	24·1	16·8

(3) Seasonal fluctuations of ruminal infusoria of sheep grazing on the veld are described. The amount of protein available in the pasture was shown to have a significant influence on the density of the infusorial population.

(4) Data are presented comparing the density and types of infusoria in veld-grazing sheep and different species of antelopes in their natural state.

(5) The digestion of maize starch within an infusorium from material *in vivo* is described. The brown glycogen-like granules formed within the foodsac and plasma of the infusorium have been shown to be *glycogen synthesizing bacteria* and not actual *glycogen granules* as hitherto accepted.

(6) The rate of digestion of starch within the rumen was shown to be the same whether infusoria were present or not. It was therefore concluded that infusoria do not accelerate the rate of digestion of starch and that they merely act as hosts to starch attacking bacteria and bacterially secreted diastatic enzymes ingested by the organism

(7) That infusoria assist in the digestion of cellulose could not be proved. It was concluded that the digestion of cellulose within the body of the infusorium is primarily due to cellulose digesting bacteria ingested by the infusorium.

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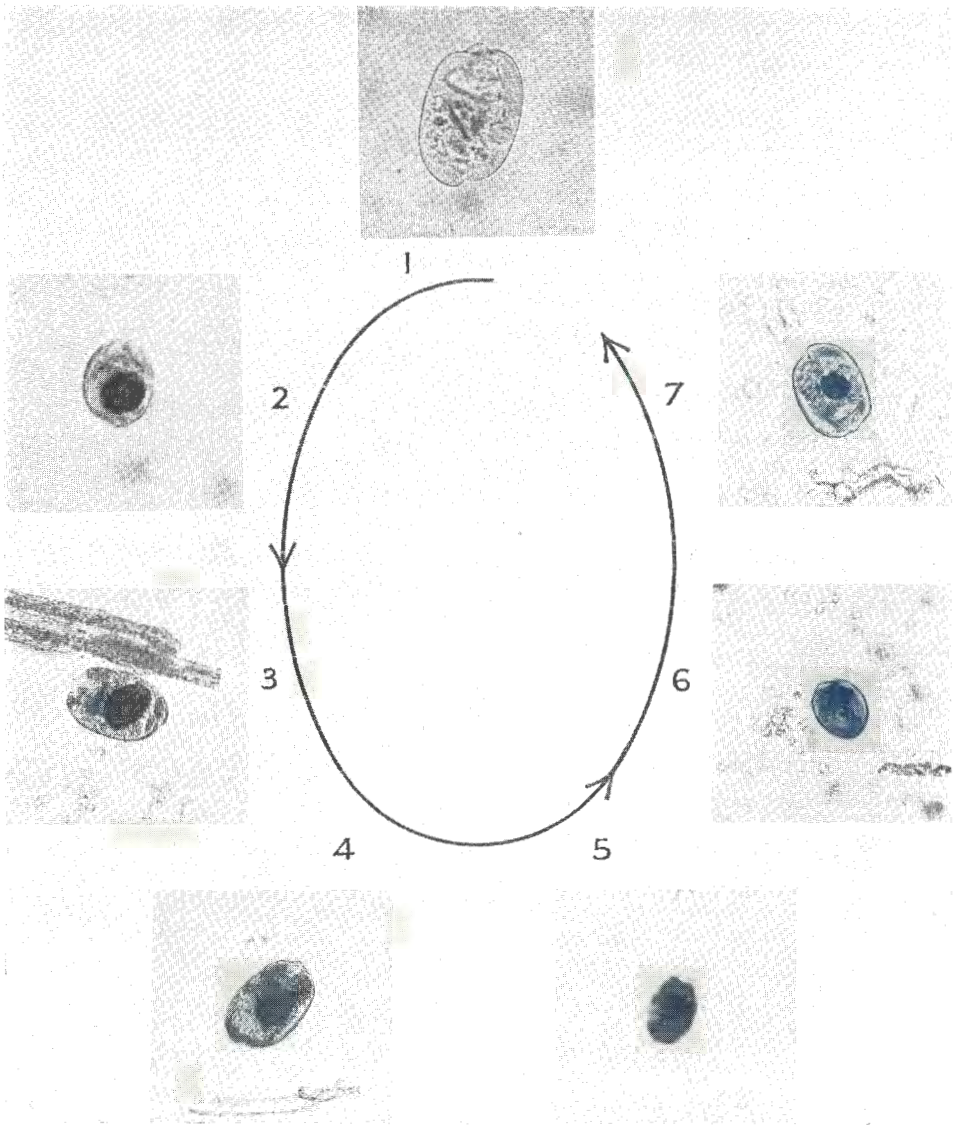
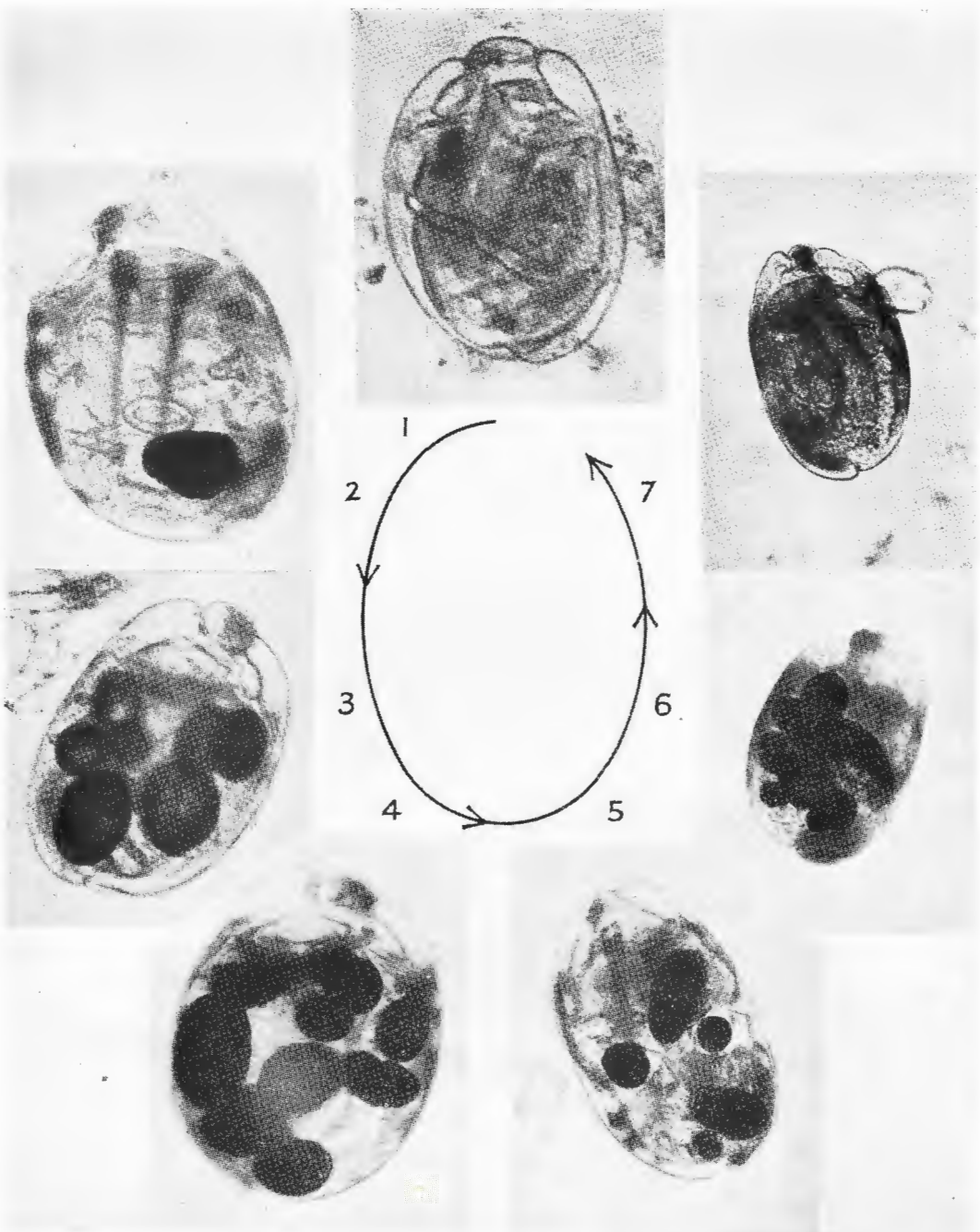
PLATE 1.



Infusorial digestion of starch: Gen. *Entodinium*.—1, Hungry *Entodinium* ×500; 2, freshly ingested starch granule×220; 3, brown granules gathering within infusorium after 1 hour×220; 4, increased brown granules after 2 hours; 5, complete obliteration of structure by brown granules—6 hours; 6, granules losing their iodophilic reaction—18 hours; 7, further advanced stage after 24 hours. At 48 hours the granules are usually translucent again.

PLATE II.



Infusorial digestion of starch: Gen. *Diplodinium*.—1, Hungry *Diplodinium* ×270; 2, freshly ingested starch granule×270; 3, brown granules gathering 1 hour after ingestion of starch grain×270; 4, brown granules increased 2 hours after feeding; 5, some freshly ingested starch granules 6 hours after initial feed; 6, brown granules masking infusorial structure after 18 hours; 7, granules cleaning up after 24 hours.

PLATE III.

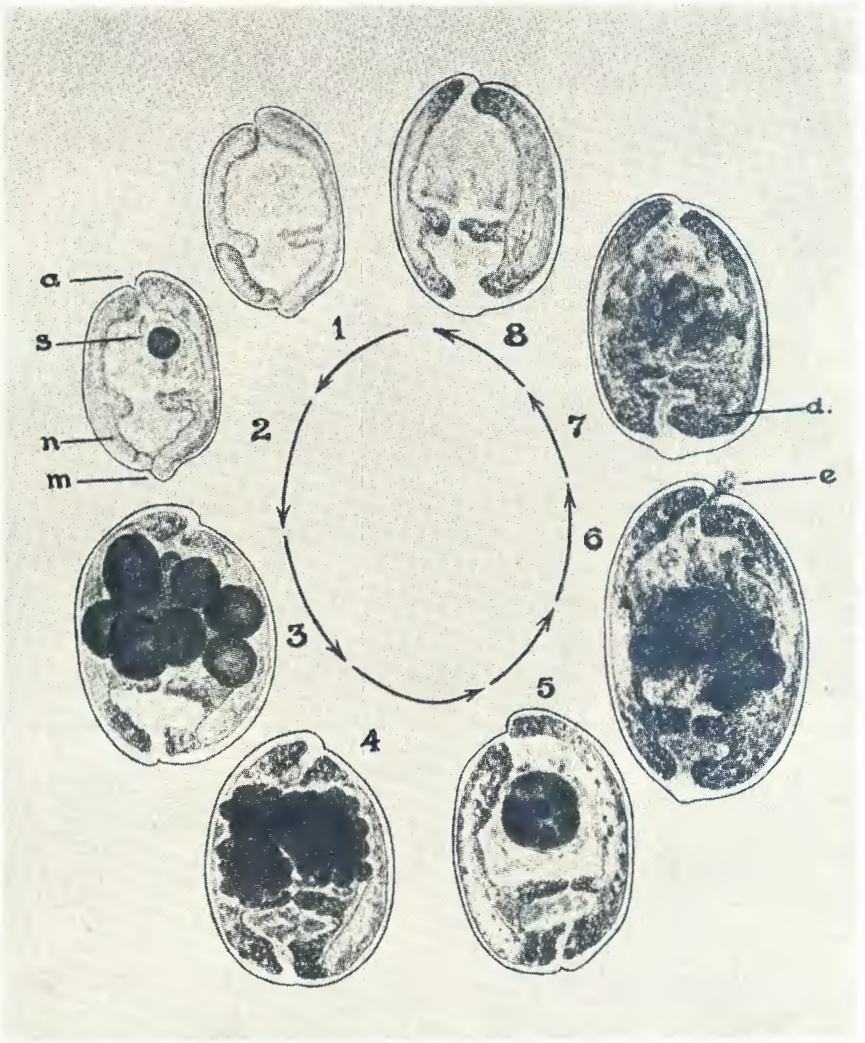


Photo of coloured plate showing digestion of starch granule within an infusorium.—Gen. *Entodinium:* a=anus; s=starch granule; n=nucleus; m=month; d=glycogen containing bacteria; e=discharge through anus.

Digestion cycle.—1, hungry infusorium containing some transparent bacteria; 2, infusorium with freshly ingested starch granule; 3, engorged infusorium showing commencement of bacterial synthesis of glycogen; 4, synthesised glycogen staining brown in the bacteria contained within the infusorium; 5, disintegration of starch granule commenced; 6, accumulated masses of glycogen containing bacteria with expulsion of some from anus; 7, complete disintegration of starch granule, leaving a dark mass of iodophilic bacteria; 8, almost complete metabolism of glycogen by bacteria.