The total gain in weight for the infusoria-free lot in 8 weeks was 156 lbs. as contrasted with 117 lbs. for the normal infected lot. Thus to date the infusoria-free lot has shown a weight increase of 133.3% as much as that of the other lot. The lambs were weighed individually and a table of individual weights each week in pounds and ounces will be published in a later paper. The experiment will continue at least until September first. The results of the experiment to date certainly indicate that, as measured by weight gains of growing animals, ruminants derive no benefit from the infusoria normally present in the first two divisions of their stomachs. We reserve our judgment as to whether or not these protozoa are a handicap until the experiment has progressed further.

Acknowledgment.—The experiments reported in this note are being made possible by grants from the American Association for the Advancement of Science and from the BACHE FUND OF THE NATIONAL ACADEMY OF SCIENCES.

THE METHOD BY WHICH RUMINANTS ACQUIRE THEIR FAUNA OF INFUSORIA, AND REMARKS CONCERNING EXPERIMENTS ON THE HOST-SPECIFICITY OF THESE PROTOZOA

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Communicated June 28, 1929

A successful method of obtaining infusoria-free ruminants for experimental purposes was recently reported (Becker,[1] 1929). After the difficulty of obtaining uninfected animals was surmounted, it was necessary to learn a number of facts concerning the life-history of these protozoa and the method by which ruminants naturally become infected, in order that we might maintain our animals azoic after they had once been rendered so. Observation by a number of workers that young calves, lambs and kids first show protozoa in the rumen and reticulum portions of their stomachs at about the time they begin to ingest hay led perhaps to the belief that the infection was obtained from the hay.

Eberlein[2] performed the first experiments in the nature of an effort to determine the source of infection. By means of a tube thrust down the esophagus of a kid he was able to obtain samples of rumen content for microscopic examination. He discovered that the kids which had not yet learned to eat hay did not harbor the rumen infusoria. After weaning, however, these protozoa were present in large numbers. Likewise, he

found that he could control the presence or absence of these organisms through manipulating the food of the host. Animals deprived of hay and put on an exclusively milk diet ceased to show infusoria. They returned, however, when the hay diet was resumed. This led Eberlein to make the oft-quoted statement that the hay, in connection with the water, is the source of the infection. He was led to the hypothesis that cysts (*Dauerformen*) were present on the hay, although he freely admits that he was unable to demonstrate such stages. He also unsuccessfully attempted to free the hay of this hypothetical source of infection by means of sterilization with heat. His failure led him to state further that it was practically impossible to kill the cysts. His attempts to cultivate rumen infusoria from the hay were also failures, although he mentions that Certes claimed to have cultivated similar organisms from dead leaves. The reason for Eberlein's conclusions is clear to us now. As will be explained later on, he probably had not completely disinfected his kids by the milk diet, although his microscopic examinations had furnished him grounds for the belief that he had done so.

Günther[3] defaunated his animals with hydrochloric and citric acid—at least he considered that he had done so. He followed the administration of acid-filled paraffin-coated gelatin capsules with feedings of cooked oil meal, carrots, sugar beets, etc., and he gave boiled water for drink. It is very doubtful if his method was actually effective in killing off all the infusoria, for they will not develop in an animal on this diet; since, as shown by Trier, chlorophyll and cellulose are essential for the growth of these organisms. The infusoria returned when Günther again put his animals on a hay diet. He also became committed to Eberlein's hypothesis that the hay was the source of infection. He interpreted the fact that he could not infect his supposedly infusoria-free animals by means of hay cooked for three hours to mean that the heating had killed the cysts. To us it signifies that the cooking had rendered the hay an unsuitable medium for the multiplication of the few infusoria still present in the rumen, but this exact thought does not seem to have occurred to him. Like Eberlein, Günther was unable to demonstrate the resistant forms in which he believed.

Liebetanz[4] claimed that he had discovered, and indeed he figured, the cysts (?) on the hay which had eluded Eberlein and Günther. Like them, however, he was unable to cultivate rumen protozoa from the hay. Unfortunately, some of his cysts, according to Braune,[5] were proved by Günther to be plant forms (*Uredineensporen*); while others, Braune declares, are worm eggs. Braune, who looked further for cysts, states, "Aus meinen bisherigen Angaben ist hervorgegangen, das es mirnicht gelungen ist, irgendwelche Dauerformen der Protozoen aufzufinden." His animal infection experiments, in which he found that the hay contained the source

of infection, are open to the same criticisms as those of Eberlein and Günther.

"Regarding the Ciliata found in the rumen and reticulum of sheep and cattle," writes Fantham,[6] "it is interesting to record that species of *Entodinium* and *Diplodinium* may be found on wet grass and in aqueous washings of fresh grass and even of dried grass (fodder) from sheep runs and pasture. The firm cuticle of the Ciliata sufficiently preserves the organism during such exposure (*This we doubt!*). Sheep and cattle are infected with the ciliates mentioned while eating grass and hay." We wish to state that we believe that it is not impossible that such forms were found just as stated above, provided the animals had grazed over the grass just before the samples were collected for examination. We have found the ciliates in the mouths of sheep. The ciliates are very short-lived, however, outside of their host.

Becker and Frye[7,8] (1927 and 1929) examined the feces of forty calves on a hay diet in a special search for cysts of the stomach infusoria. They were able to identify all the protozoan cysts which they found as those of *Endamoeba bovis*, *Giardia bovis*, *Eimeria* of two species and *Buxtonella sulcata*, a ciliate inhabiting the caeca of cattle. No forms which could be interpreted as cysts of rumen infusoria were found.

Scheunert[9] (1927) has best expressed the present status of our knowledge regarding the mode of transmission of ruminant protozoa. He says, "Der Ursprung dieser kleinen Lebewesen ist trotz zahlreicher Untersuchungen (Eberlein, Günther) noch nicht mit Sicherheit festgestellt, dürfte aber wohl zweifelsohne in der Nahrung der Tiere, und zwar nach Liebetanz im Heu zu suchen sein."

Our experience has been that an animal which is once freed of all of its rumen protozoa will not become infected from ingesting unsterilized hay, grass, feces of infected animals (either dried or fresh), grain, or water. It will not become infected if it is kept in quarters occupied for a long time by infected animals. These statements will prove rather startling to some. We believe that Eberlein, Günther and Liebetanz fell down in their infection experiments through incomplete defaunation of their goats because of the imperfections in the methods they employed. We record below some of our experiments. All animals were kept isolated from contact with infected animals except where otherwise stated.

Goat No. 3. Defaunation process completed Nov. 23, 1928. Given hay steamed one-half hour at atmospheric pressure and unsterilized grain daily. Remained free of infusoria until Jan. 23, 1929, when it was purposely infected. The attempted sterilization of the hay was altogether unnecessary because there are no cysts of ruminant infusoria on the hay, as we have since learned.

Goat No. 4. Same record as Goat No. 2.

Goat No. 5. Defaunation process completed Dec. 8, 1928. Received unsterilized alfalfa hay and grain mixture daily up to present writing. Given through a stomach tube 5 grams dried goat feces from an infected goat daily for 8 days (Jan. 12 to Jan. 19). Given green corn plants from greenhouse daily from Jan. 19 to Jan. 24. On Jan. 26 the goat was given through a stomach tube 10 grams fresh feces collected from six sheep. On Feb. 25 a handful of fecal pellets from an infected goat was put into the drinking water. These were left there until March 9. The water drunk was renewed daily but the pail was not cleaned out. From April 13 to May 20 the goat spent part of each day in a movable cage on green grass. From April 27 to May 6 the animal was given, either through a stomach tube or by drenching, a gallon of dried stomach contents of a goat infected with *Diplodinium hamatum, Entodinium longinucleatum* and *Isotricha prostoma*. This material was mixed up in water before administration. On May 6 and 7, 15 grams of mortarized dried cow feces from a manure pile were fed in the grain. On May 26 the goat was put into a 5 ft. $\times$ 9 ft. pen which had been occupied until the evening of May 25 by two infected goats, one for two months and the other for thirteen days. On June 15, a total of twenty-one days, the goat was still free of protozoa. Throughout all the experiments unsterilized water was given for drink.

Microscopic examinations of samples taken from the rumen on Dec. 9, 28, 30, Jan. 5, 11, 19, 24, Feb. 7, 13, 25, March 15, 26, April 14, 27, May 5, 12, 20, 31, June 10 and 15 were all negative for infusoria.

The foregoing account of our attempts to infect this infusoria-free goat includes almost every conceivable method except either direct or indirect contact with secretions from the mouths of infected animals. We believe that this experiment alone has shown that there are no sources of infection in hay, water, feces, or even dried stomach contents of infected animals. It should be mentioned that the flagellates *Trichomonas* and *Callimastix* and a smaller one, perhaps *Sphaeromonas*, returned in small numbers in about three weeks after the disinfection. It seems impossible to permanently exclude these flagellates.

Goat No. 6. Sterilization completed Jan. 16, 1929. Unsterilized alfalfa hay and a grain mixture were fed throughout the whole experiment, either as the sole feed or as a supplementary ration. The drinking water was offered to the animal just as it came from the tap. On March 9 about thirty grams of fresh feces from two calves mixed with water was poured into the rumen through a stomach tube. Powdered dried goat manure was put in with the grain mixture at frequent intervals. The goat in the stall next to this one was infected with a number of species of infusoria. The goats were made to trade stalls at frequent intervals, but no mouth to mouth contacts were allowed. Feed boxes and water

buckets were traded at the same time, so that each goat kept its own. A few ounces of the dried stomach contents of an infected goat were fed on April 29 and May 3. Part of each day from April 13 to May 6 was spent grazing on a grass plot on the college campus. As reported below, some material containing horse infusoria was also fed to this animal.

Microscopic examination once every week up to May 13 showed that the goat had not acquired an infection with infusoria. This goat, like the preceding, was exposed to a wide variety of conceivable sources of infection. Yet both animals were susceptible to the infusoria; for under the proper conditions, to be described immediately, infection did take place. We find in our work that it is very easy to infect any infusoria-free ruminant with a drop of infected stomach contents.

Successful Attempts to Transmit the Infection.—Goat No. 6. On May 13 this goat put into a pen with an infected goat. It was permitted to eat from the same feed box and drink from the same water bucket that the infected goat used. No microscopic examination was made until May 25, when it was found to have become heavily infected with the same protozoa as its cage mate; viz., *Isotricha prostoma, Diplodinum hamatum, Entodinium simplex* and *E. longinucleatum.* Since the infected goat was very hostile toward the one which was not infected when first put into the cage, it is almost certain that no mouth to mouth contacts, as by licking, took place. Thus this goat, which had been free of infusoria since Jan. 16, became infected by eating from the same feed box and drinking from the same pail with the infected goat.

Goat No. 5. On June 15 an experiment to test out further the mouth-contamination theory was begun. This goat, which had been free of infusoria since Dec. 8, 1928, was put into a 5 ft. $\times$ 9 ft. cage to graze on the grass with an infected goat. The two animals were not permitted to drink out of the same container. No licking or other mouth-to-mouth contact was observed, although this might have taken place. The cage was moved from place to place at frequent intervals, so that the two animals grazed over the same area together. They were also permitted to eat grain out of the same feed box. On June 22 a microscopic examination of the rumen contents was negative for infusoria, but on June 24 *Entodinium* was present. Licking each other's mouths was never noted, but it is impossible to say that this did not occur, since the goats were not under constant observation.

The conclusion which we draw from our experiments is that the infection is transmitted through saliva which may moisten the feed while the animals are eating. That such moistening does take place is easily demonstrable in the hay boxes from which our lambs feed, though part of the moisture is from the nasal secretions. It is possible also that the infection could take place through licking, or through the drink, but we

have not demonstrated this. The question may arise so to whether there are actually protozoa in the saliva of a ruminant.

One of us (E. R. B.) on June 12, 1929, took a few drops of saliva by means of a pipette from far back in the mouth of seven infected lambs which had been observed to ruminate a few minutes before. In the sample from each lamb *Entodinum* was demonstrated by means of careful search under the low power microscope. Our transmission experiments which indicate that infection takes place by means of contamination from the mouth thus became easily intelligible.

It is usually presumed that the infusoria of various species of ruminants are more or less non-specific for their hosts. We have demonstrated this to be true by infecting two azoic goats with *Diplodinium hamatum*, *D. bursa*, *Entodinium longinucleatum*, *Entodinium simplex* and *Isotricha prostoma* from a calf. *D. bursa* died out for some reason after about two months, but the rest persisted. We later infected a lamb with the protozoa remaining in the goat.

Our attempts to infect the rumen of an azoic goat (Goat 6, while infusoria-free) with certain species of infusoria in the colon contents and feces of horses were unsuccessful. We identified in the materials from the horse live specimens of *Cycloposthium bipalmatum*, *C. scutigerum*, *Blepharocorys angusta*, *B. curvigula*, *B. jubata*, *Didesmis quadrata*, *D. ovalis*, *Bundleia postciliata*, *Tripalmaria dogieli*, *Triadinium caudatum*, *T. galea*, *T. minimum*, *Ditoxum funinucleatum*, *Tetratoxum unifasciculatum*, *Spirodinium equi*, *Charon equi*, *Paraisotricha colpoidea* and *Allantosoma intestinalis*. Although we checked up on the goat's rumen contents for weeks after feeding these infusoria through a stomach tube, no infection took place. This shows that the horse infusoria will not develop in the stomach of ruminants, even though competition with the natural fauna of this region is eliminated by its complete removal.

This experiment is of further interest because of the close relationship between two of the genera of horse and ruminant infusoria. *Charon equi* (lately described by Hsiung) is very similar to *C. ventriculi* Jameson from the cow. Dogiel has described a member of the genus Blepharocorys, *B. bovis*, from cattle, but there is a possibility that this is a synonym of *Charon ventriculi*. The horse infusoria include a number of species of this genus. Further, *Bundleia postciliata* has but recently been removed from the genus *Buetschlia*, which is found in ruminants. So we see that in spite of morphological resemblances these ciliates maintain distinct physiological relationships with their hosts which preclude their growing in widely different hosts or locations.

Conclusions.—1. The source of the stomach infection of ruminants with infusoria is not naturally in the hay, grass, water, grain, feces, or even in dried stomach contents of infected animals.

2. Our experiments indicate either that cysts or resistant forms of these infusoria do not exist, or that they are extremely rare, and formed only under exceptional conditions.

3. The infection is spread from one animal to another by mouth contamination. To become infected through the food an animal must eat food contaminated with infected saliva before it dries, for drying will kill the infusoria. The infection may perhaps also be spread by licking, or drinking contaminated water; but we have not proved this. We have excluded absolutely the water in one experiment, and it is extremely improbable that licking occurred in either of the two in which we succeeded in obtaining infections under natural conditions.

4. Living representatives of stomach forms may be found in the mouths of infected animals.

5. Certain infusoria of the stomach of the goat, cow and sheep show no host-specificity within these three host species.

6. The infusoria of the colon and feces (which come from the caecum and colon) of horses will not develop in the rumen of goats.

Acknowledgments.—The work reported in this paper was made possible by grants from the American Association for the Advancement of Science and from the BACHE FUND OF THE NATIONAL ACADEMY OF SCIENCES. The writers are also indebted to Mr. Ralph C. Everett for assisting them in handling the goats.

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