METHODS OF RENDERING THE RUMEN AND RETICULUM OF RUMINANTS FREE FROM THEIR NORMAL INFUSORIAN FAUNA

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The protozoan fauna which is peculiar to the rumen and reticulum of the ruminant stomach was first discovered by Gruby and Délafond (1843). The general failure to find uninfected animals has led to the belief of subsequent investigators that all normal ruminants which receive a diet containing chlorophyll and cellulose harbor Infusoria belonging to the families Isotrichidae, Buetschliidae, or Ophryoscolecidae and a number of species of small flagellates and amoebae. Not every animal harbors all these groups, but representatives of the family Ophryoscolecidae, belonging to the genera *Entodinium* and *Diplodinium* are usually present. One investigator, Liebetanz,[1] reported that out of 175 ruminants of various kinds which he examined at the slaughter-house there were three goats in which no Infusoria were present. The writer made repeated examinations of the stomach contents of an isolated experimental calf without finding any Infusoria. Flagellates of the genus *Callimastix*, however, were present. Some of the Infusoria of ruminants, particularly the Ophryoscolecidae, feed upon particles of hay and starch, as well as upon bacteria. This has led to a belief on the part of many workers that these Infusoria are in some way useful to their hosts. The flagellates are considered to be of no particular importance because they do not ingest hay and starch particles.

The writer recently became interested in the problem of the physiological rôle of these Infusoria in their host. The obvious method of determining the effect of the activities of the protozoa would be a comparison of the digestion of food of known chemical composition in infected and "azoic" animals. It was apparent that there are two possible means of obtaining azoic animals. One is to bring them up from birth so as to prevent infection; the other is to defaunate animals obtained from the farm where, it is safe to assume, they have become infected. The first method would not only be time-consuming, but by attempting it one would run the risk of having his animals becoming infected, since little is known about the transmission of these Infusoria from host to host. For these reasons it was decided to attempt to sterilize animals already infected as practically all ruminants become after they commence to consume grass and hay. It was learned from the literature available that

three investigators had previously worked on methods of defaunating ruminants, with varying results.

Eberlein[2] reported that he had effected a conditional (*bedingte*) disinfection of the stomach of one kid by the administration of 0.05 gr. of corrosive sublimate in 200 cc. water. He describes the ill effects produced by the poison, and adds to his account the statement that the peculiar sensitivity of ruminants to sublimate is very disturbing. Whether he obtained complete and permanent freedom of the stomach from Infusoria or not, the method cannot be recommended on account of its attendant dangers. The same author also found that when he took the kids off of a hay diet and placed them on a diet of milk only, he was unable to find any more ciliates in the rumen. Very soon after the feeding of hay was resumed the protozoa returned.

Günther[3] administered to animals which had been starved for several days from ten to twelve paraffin-covered gelatin capsules holding two grams of either hydrochloric or citric acid during a two-day period. He then fed his animals cooked oil meal and boiled water followed by potatoes and carrots. The Infusoria will not develop in an animal on this diet anyway, so it will never be known if he effected a complete removal of the protozoa.

Liebetanz[1] tried the administration of hydrochloric and acetic acids by both capsule and the stomach tube methods with disastrous results to his goats. Then he conceived the idea of administering acetic acid solution through a cannula inserted directly through the body wall into the rumen. The goats had been starved for three days before the acid treatment. He apparently achieved a complete disinfection of young goats by this method but he admits that in older animals with a better developed rumen and reticulum the method presents great difficulties.

The writer gave the direct method of Liebetanz a thorough trial with rather unsatisfactory results, which will be discussed in a later paper. In two cases, however, half-grown goats were successfully defaunated. No successes were attained with adult goats. These successes did not offset numerous failures to effect complete removal of the Infusoria and fatalities to four goats and a large calf. Thymol-chloroform, lactic acid, and corrosive sublimate solution given by the rumen cannula method proved unsatisfactory.

The following method, which was developed only after considerable experimenting, proved successful in every attempt. Up to the present time it has been used by the writer on six different occasions, two times in each of two goats about 9 to 10 months old and in two adult goats. First, the goats are given absolutely no food for three days, but water is always kept before them. Then at the end of 72 hours the jaws are held apart by an improvised speculum made of a piece of pine wood (2 in. x 2 in.)

with a hole in the middle. A rubber horse catheter, well soaped, is passed through this hole and down the esophagus into the rumen. It is then necessary to put the free end of the tube up to the ear and listen for breathing in order to determine for sure that the other end of the tube is not in the trachea. Then 50 cc. of two per cent copper sulphate (I used the clear crystals of the commercial grade) mixed with about a pint distilled water is poured through a funnel into the tube leading to the rumen. Twenty-four hours later the same treatment is given, the animal having received no food meanwhile. About four hours later the animal is offered a small amount of alfalfa hay and some whole oats. He commences to eat greedily, but does not eat much. The next day he eats little if any. The copper sulphate seems to produce a loss of appetite. Henceforth the appetite improves daily, until by the end of the fifth day after the second administration of the poison it becomes almost normal.

The animal suffers no permanent ill effects, and in a few days is as well and lively as ever. One can check up on the presence or absence of Infusoria by microscopic examinations of rumen samples pumped from the stomach through the horse catheter mentioned above. If the protozoa do not return by the end of two weeks the animal will remain free of them unless a reinfection takes place.

A large number of preliminary trial experiments resulted in failure to disinfect the rumen of protozoa. These were usually for one of two reasons: either the starvation period was too short, or the dosage of copper sulphate was too light. It is better to give a little more than the above stated amount than to give less. Even a two-day starvation period before the first treatment has been shown to be insufficient, and feeding the animal hay or grain before the second treatment will cause failure.

Experiments with varying amounts of copper sulphate ground to a powder in a mortar and mixed thoroughly in the grain ration will reduce the numbers of protozoa but will not completely free the rumen of them. Presumably the effect of the copper sulphate is lost by the formation of copper proteinates.

If the disinfected animals are kept away from other animals and given ordinary unsterilized alfalfa hay and a grain mixture, the Infusoria will not return. I have one goat which has been free of Infusoria for about six months. In some way the flagellates *Callimastix* and *Trichomonas* find their way into the rumen again within two or three weeks, even when the animal gets sterilized feed. To keep them entirely out would seem to be a practical impossibility. It is not of much importance for my purposes to do so, for it is the Infusoria which feed upon the hay and grain elements within their host. The bacteria of the rumen soon return again, although it is not certain that the nature of the flora may not be at least temporarily changed.

The above-described method of freeing goats from protozoa will be tested out on sheep and cattle, where it should also be effective. In the goat it has the three primary requisites of any good method; viz., safety, certainty and simplicity.

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[1] Liebetanz, E., *Arc. of Prot.*, **19**, 1910 (19–80).
[2] Eberlein, R., *Zeit. f. Wiss. Zoöl.*, **59**, 1895 (233–304).
[3] Günther, A., *Zeit. f. Wiss. Zoöl.*, **65**, 1899 (529–572).

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*MONATOMIC IODINE AND MOLECULAR HYDROGEN**

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In 1884 Amato[1] showed that a mixture of hydrogen and chlorine in glass at $-12°$ could be exposed to the direct rays of the sun for hours without any appreciable reaction taking place, although the two gases will combine explosively if exposed to bright sunlight at ordinary temperatures.

About fourteen years later this suggested to Kastle and Beatty[2] the possibility that sunlight would cause hydrogen and bromine to react if the experiment were tried at a higher temperature. A number of qualitative measurements were made at 196°, the bulbs, containing an excess of hydrogen, being placed in the vapor of boiling orthotoluidine. In the dark the reaction is exceedingly slow, and practically negligible in a three-hour run. In the sunlight the reaction takes fairly rapidly. In the first series the bromine was practically all gone at the end of an hour, as judged by the eye. In the second series the bromine had reacted practically completely in ninety-five minutes. In the third series an estimated fifty per cent of the bromine reacted in the first fifteen minutes and practically all the rest in the next thirty minutes. In other words the bromine is converted practically completely into hydrobromic acid in from forty-five to ninety-five minutes.

The differences in the time for practically complete disappearance of bromine were due to differences in the intensity of the light. The bulbs