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Studies on the Alimentary Tract of Merino Sheep in South Africa. IV.—Description of Experimental Technique.

By J. I. QUIN and J. G. VAN DER WATH, Section of Physiology, and S. MYBURGH, Section of Biochemistry, Onderstepoort.

Introduction.

In the first two papers which appeared under this series (Mönnig and Quin, 1933, 1935), methods were described whereby the process of deglutition and the closure of the oesophageal groove was studied in Merino sheep. Various selected substances were dosed on to the back of the tongue of experimental animals and followed within short intervals by some indicator powder such as red oxide of mercury. All animals were slaughtered immediately after dosing, and the forestomachs, abomasum and intestines opened and examined for the presence of the indicator powder. By this procedure considerable information was gained as to the peculiarities of the oesophageal groove reflex and the route followed by materials in their passage along the digestive tract under varying circumstances.

In the third paper (Besselaar and Quin, 1935), the results were discussed of experimental bowel anastomosis in sheep. By this method, measured lengths of either large or small intestine were excluded from function through a short circuiting of the bowel. This afforded a means of assessing the significance of a decrease in the digestive and absorptive surface to general body economy.

As various other aspects of ruminant digestion are also under investigation, the present paper is devoted mainly to a description of some further experimental methods which the authors are adopting in the study of this subject.

Digestion in ruminant animals is admittedly a more complicated and more protracted process than that occurring in most of the other species of mammals, and consequently our knowledge in this respect is less complete than that concerned with the digestion of man or dog. Thus, very little is as yet known about the degree and significance of the predigestion of foodstuffs occurring within the forestomachs as a result of bacterial and possibly other types of fermentation when compared with the true digestion which follows in the abomasum and upper levels of the small intestine. Similarly little is known about the activity of the digestive juices secreted from the true stomach and intestines of ruminants in comparison with those, for example, from the dog, although in most text books it is assumed that the processes are identical. With regard to the fundamental factors concerned in absorption from, and excretion into the digestive tract of ruminants our knowledge is equally incomplete.

It was for the above reasons, therefore, that experiments were devised primarily with the object of obtaining a clearer insight into the functioning of the different levels of the digestive tract of ruminants. For this purpose well-grown, adult, wether Merino sheep were selected in the majority of experiments, although a few bovines were also included in some aspects of the work carried out.

Experimental Procedure.—The Creation of Bowel Fistulae.

As an experimental method, the extensive use of bowel and salivary fistulae was first introduced by Pavlov in his classical work on digestion which was conducted mainly in dogs. By this method, he was able to gain an insight into the various phases of digestion which up till then had been largely shrouded in mystery. To-day the fistula method is well established as a means of investigating the digestive tract, although it is still largely confined to such animals as the dog. Thus, in ruminants it has so far only found limited application except as a means of studying the motility of the rumen and other forestomachs. In such cases large open fistulae are usually created by uniting the incised rumen with the abdominal skin in a flank operation. Wester (1926) exclusively used this method in his well-known work on the motility of the forestomachs and the closure of the oesophageal groove in cattle. Likewise did Schalk and Amadon (1928) and also Bergman and Dukes (1926) utilize this procedure in their researches. Recently, Krzywanek and Quast (1936), working on sheep, reported good results by using the improved double method of Brüggemann (1935) as based on the original device suggested by Morat.

Apart from the above-mentioned fistula methods, various other procedures have from time to time been adopted. Thus Czepa and Stigler (1929), working on goats, followed the onward movement of contrast mixtures through the forestomachs and abomasum by means of Röntgen ray photography. A similar method was adopted by Magee (1932) in his studies.

A third method which has found favour with some workers consists in the opening of the abdominal cavity of narcotised experimental animals in a bath of saline raised to body temperature. This allows of a close visual inspection of the bowel movements as well as their recording by means of cinematography. Thus far Mangold and Klein (1927), and more recently Dukes and Sampson (1937), all working on sheep, have recorded the results obtained by this procedure.

The method adopted in our experiments consisted in the creation of "closed" fistulae at various levels of the digestive tract in contrast to the "open" fistula method as commonly used on ruminant animals. For this purpose various types of cylindrical fistula tubes, mounted with a flange at one end, have been experimented with, the shape and size of the tube depending on whether it was intended for use in the rumen, abomasum, small intestine or large intestine. Of the metal tubes used, both the aluminium, and the chromium plated brass tubes proved rather disappointing since after being in the bowel for some time, sloughing frequently followed corrosion of the tubes either by the acids or alkalis elaborated in the tract. At other times, again, electrolysis would result in the deposition of materials at the internal opening of the tube thereby blocking it up. Thus far the tube which has given the minimum amount of trouble, especially as used in the rumen and caecum, is one turned up in a lathe from a cylindrical block of ebonite and provided with a wide flange at one end. Such tubes, not being subject to corrosion and electrolysis, are found to remain in their proper position in the body more or less indefinitely (see photo No. 1—ebonite tube on extreme right). For use in the rumen, a tube 6 cm. long, with a bore of 1·5 cm. and a circular flange of 4 cm. diameter, has so far given very satisfactory results. Correspondingly smaller tubes of the same pattern are used in the abomasum and the caecum. For the small intestine, much narrower tubes are employed, these being mounted with an oval boat-shaped flange at one end. In each case a slight hollowing out of the flange, thus presenting it with a turned-up appearance, has been found to be advantageous.

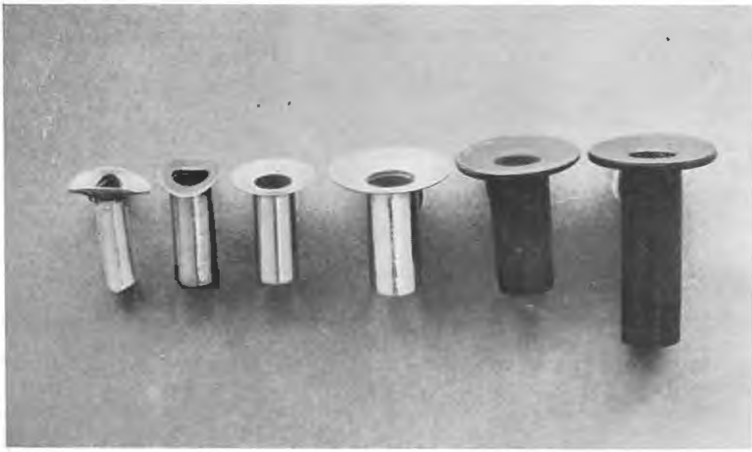

Photo No. 1.—Bowel fistula tubes of different types.

Insertion of the Fistula Tube into the Bowel.

During a pre-period of 4 to 6 weeks, selected animals are penned off individually and kept on a constant ration of 360 grams crushed yellow maize and 300 grams hay daily. All animals are completely starved for 24-48 hours prior to operation, during which time the abdominal skin is clipped and shaved. The left flank is thus cleaned when a rumen fistula is to be inserted, whereas for all other intestinal fistulae the right flank is utilised. Abomasal fistulae, however, are brought out in the mid ventral position about 8 cm. posterior to the Xiphoid cartilage.

In each case general anaesthesia is induced through slow intravenous infusion of 10 per cent. choral hydrate in saline, narcosis being subsequently maintained with light ether inhalation, should this be necessary. Full aseptic precautions are observed throughout the operation, which comprises a laparotomy through either flank about 5 cm. behind and parallel to the last rib except in the case of an abomasal fistula, where a mid ventral opening is resorted to. For the creation of a rumen fistula the dorsal aspect of the dorsal ruminal sac is invariably selected. This part of the rumen, after being withdrawn through the abdominal wound as far as possible, is firmly caught up in a pair of long curved bowel forceps guarded with rubber tubing on both limbs. Using No. 1 gut on an eyeless needle, an oval purse string pattern is sutured through the muscular layer of a small selected part of the ruminal wall. See photo No. 1a.

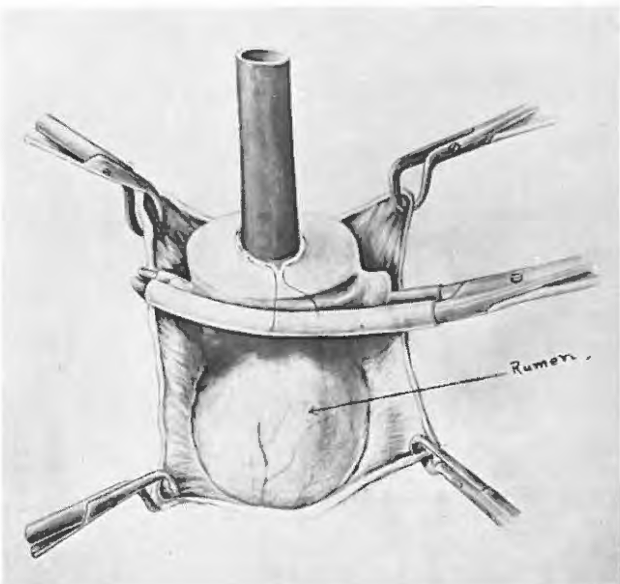

Photo No. 1 (*a*).—Diagrammatic sketch of a stage in ruminal fistula operation.

The ebonite tube, sterilised by boiling, is mounted with a short stout piece of rubber tubing at its thin end prior to its insertion in the rumen. A short slit is made through the rumen in the centre of the purse string suture through which the flange of the tube is then passed by slight stretching of the ruminal wound. This is followed by invagination of the lips and a tightening of the suture. Usually a second purse string suture is applied just above the first. After that, the free end of the tube is passed through a small slit made in the omentum which in this way is made to cover the ruminal incision. Next, a point on the skin is selected high up in the angle formed between the last rib and the transverse processes of the lumbar vertebrae. A stab wound is made at this point into the peritoneal cavity. By means of two pairs of long artery forceps passed through this wound, the rubber mounting on the fistula tube is securely gripped and the tube withdrawn through the stab opening as far as possible. This ensures a close contact between the rumen and the parietal peritoneum immediately around the flange of the tube. This contact is subsequently maintained by a collar of cotton wool tied down tightly round the tube between the rubber mounting and the skin. This is followed by closure of the laparotomy wound by three layers of continuous gut sutures one layer each for the peritoneum, muscle and skin respectively. The only dressing applied to the fresh wound is a thin film of collodion-iodoform. Healing in practically every instance follows by first intention. A firm circular adhesion

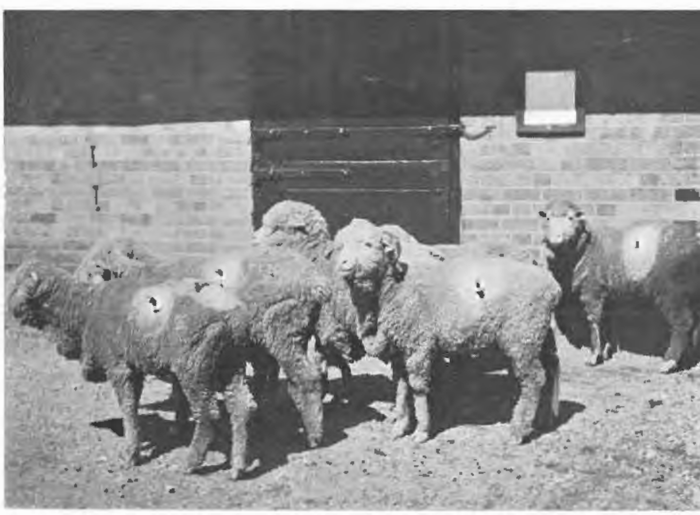

Photo No. 2.—Sheep with "closed" ruminal fistula.

is thereby established round about the fistula tube, usually no more than 6 cm. in diameter, uniting the rumen, omentum and parietal peritoneum within a few days. For the first three or four days following the operation, animals are usually kept on a light diet of green feed after which they are immediately returned to the standard laboratory ration of lucerne hay and maize, all animals being fed and watered individually in single pens. The tubes are kept closed by tight-fitting rubber stoppers inserted into the rubber mountings stretched over the ebonite tubes. Apart from periodic cleaning up of the outside of the tube, which may become slightly soiled, no other attention is required. Such tubes usually remain in position for an indefinite period. Thus several sheep carrying tubes for two years and over are included in the flock of fistula animals. (See photos Nos. 2 and 3.) Photo No. 4 is that of a bovine with an open ruminal fistula.

Photo No. 3.—Sheep with closed abomasal fistula.

Photo No. 4.—Bovine with open ruminal fistula.

With regard to the insertion of tubes into the intestines, an essentially similar technique is followed except for minor modifications. The intubation of the caecum is generally quite satisfactory, the tube remaining in position very well. In the case of the small intestines, the walls of which are extremely thin in sheep, only small tubes can be accommodated. Even so there is a tendency for such tubes to slough some weeks to some months after their insertion, although the fistula opening in the skin may remain patent much longer. Up to the present, however, our main attention has been devoted to the study of rumen function and hence to the creation of rumen fistulae. All animals are weighed weekly and weight curves plotted. From these data and from the general appearance of the animals, it may be stated without hesitation that the nutritional condition is in no way adversely affected by the presence of the tube in the rumen.

Thus far various aspects of rumen function have been investigated on a small flock of 15 to 18 sheep with ruminal fistulae. It is now intended to describe in some detail the technique followed in the study of each of these phases.

(a) *Kymographic Recording of Ruminal Movement.*

For this purpose the usual balloon and tambour method was at first so modified as to allow easy registration at any selected pressure level. Of the different balloons tried, the ordinary round or oval toy balloon, with a capacity of 60 c.c. at 10 mm. Hg. pressure, was found to yield satisfactory results. One end of a 15 cm. length of thin glass tubing is inserted into the neck of the balloon and this securely tied down on to the tube by a strand of cotton. To allow insertion into the rumen, the deflated balloon is first rolled up so as to form a small scroll which, with the help of a thin probe, is then easily pulled through the fistula tube. By gently blowing into the glass tube attached to the balloon, the latter thus becomes unfolded in the rumen, immediately below the flange of the fistula tube.

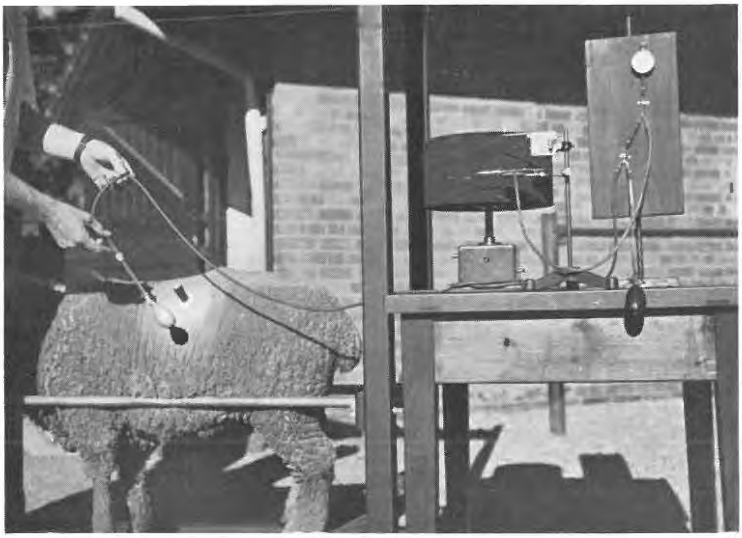

Photo No. 5.—Apparatus for recording ruminal movements. Balloon and tonometer method.

By means of a length of thick-walled rubber tubing, the balloon is connected up with a spring tonometer mounted on a wooden board. By using suitable Y piece connections, this again is brought into communication with a pressure bulb of the type used for inflating the arm band of a sphygmomanometer. From here another length of rubber tubing leads on to a membrane tambour mounted with a suitable writing lever so placed as to record movements on a slow moving clock drum. (See photo No. 5.)

By means of the pressure bulb, the whole system can be inflated to any selected pressure level as indicated on the dial of the tonometer. From experience gained, an initial pressure of 10 mm. Hg. in the balloon and other parts of the recording system was found to be suitable for work on the rumen, and hence this standard adhered to throughout. At this pressure, the whole recording system is extremely sensitive, even minute respiratory changes being clearly registered. Moreover, the actual positive pressures developed in the rumen under varying conditions could be accurately followed. By placing the animal in a specially constructed crush pen, continuous recording of movements, even over the whole day, could be carried out, without interference to the feeding or watering of the animal. Likewise, movements from other parts of the digestive tract such as the abomasum and the caecum could be registered through fistulae in these organs and the use of slightly smaller balloons. As a routine measure all movements are recorded and counted over periods of five minutes at a time, which for the sake of convenience is thus regarded as the unit of recording. In photo No. 6 specimen graphs are shown as obtained from the rumen, abomasum and caecum by this method.

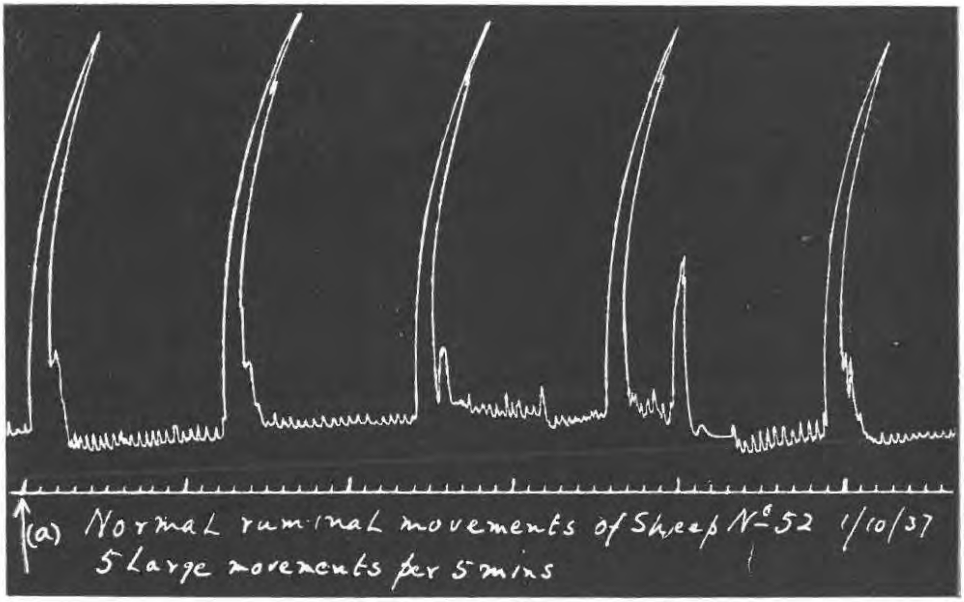

Photo No. 6.—Specimen graphs of rumen.

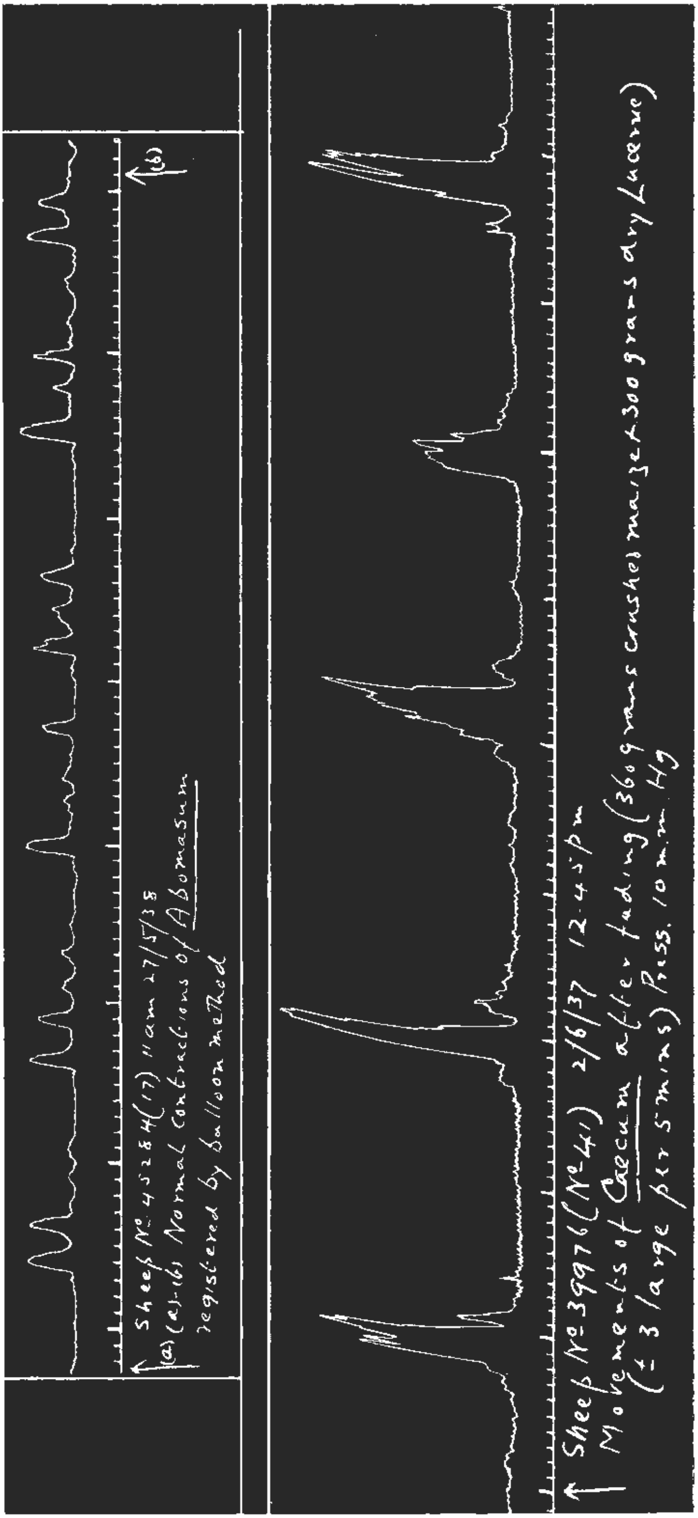

Photo No. 6 (a).—Specimen graphs of abomasum (above) and Caecum (below).

By the above balloon method it is, however, very difficult to obtain a clear insight into the maximal and minimal pressures developed with an organ such as the rumen. Moreover such determinations are of importance in a study of the gas pressure associated with fermentation, in which, either normal regurgitation of gas repeatedly takes place through the mouth, or when disturbed may lead to bloating. Consequently it became necessary to improve the above technique so as to allow the accurate recording not only of the individual ruminal contractions but especially also of the limits of pressure associated with any change either in ruminal tonus itself or that due to the accumulation of gas within the organ.

This improvement was achieved by discarding in the first place the use of the balloon in the rumen. By connecting the apparatus directly to the rumen fistula through a short piece of glass tubing, the rumen itself was now made to function as the balloon at the one end of the system. Seeing that the fistula tube in every case is so placed that it is well above the ingesta and water level in the rumen, all changes in pressure are accurately reflected in the rest of the recording apparatus. In no case is there any leakage of gas round about the exterior of the fistula tube when the fistula wound itself is well healed and the flange of the tube drawn up snugly against the abdominal wall.

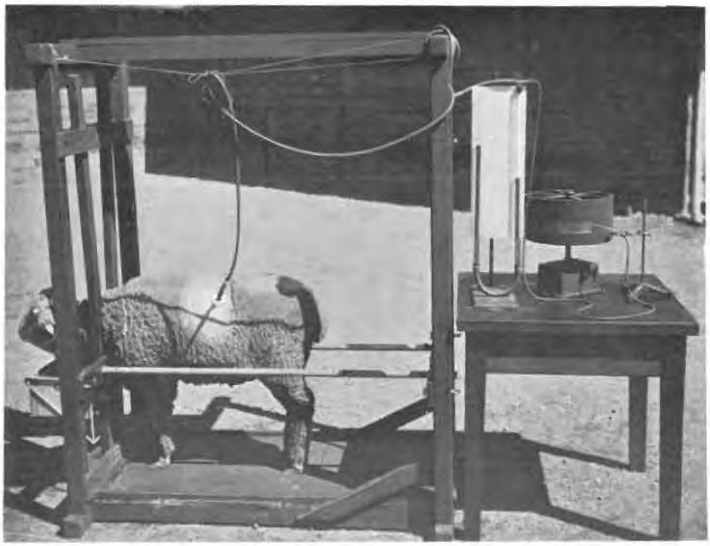

Photo No. 7.—Improved apparatus for recording ruminal movement. Water manometer method.

The second alteration made in the apparatus was to substitute a water manometer, graduated in millimeters (total capacity 220 c.c.) for the spring tonometer. By the use of acidulated water (1 per cent. HCl + methyl red indicator) in the manometer, the possibility of some rumen gas dissolving in it was thereby obviated. Photo No. 7 shows the improved apparatus fitted with the water manometer. By this method it became possible to register in minute detail all the pressure fluctuations, either on the positive or negative side, occurring within the rumen. Also by subsequently changing the pressure in the manometer to the required levels, parallel lines could be drawn on the drum so as to record the limits between which ruminal pressures had actually fluctuated. (See photo No. 8.)

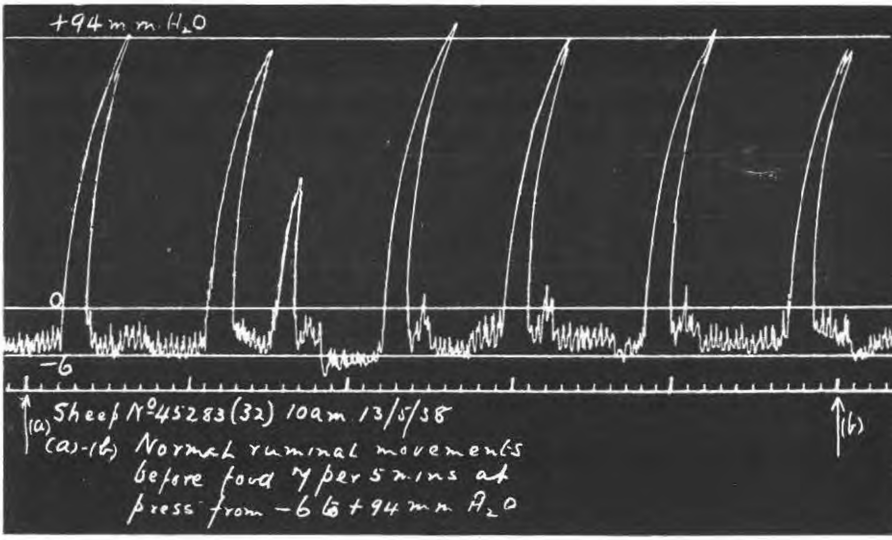

Photo No. 8.—Specimen graph of rumen.

One noticeable feature revealed by such graphs is the frequent occurrence of negative pressures within the rumen of certain animals, a more detailed discussion of which will be given in a subsequent article.

Apart from registering normal ruminal movement, the above method also allows for a detailed recording of the tonus reaction of unstriated muscle of the digestive tract (as represented by the rumen) to a wide variety of factors such as food, drink and action of drugs and other chemical substances.

(b) *Sampling of Contents from the Digestive Tract.*

In any study on digestive function, the ease with which portions of the contents can be collected from different levels of the digestive tract, constitutes a very valuable means of investigating the vital processes involved in the disintegration and subsequent absorption and re-excretion of all materials taken in *per os*. Such sampling is readily achieved through fistulae inserted at suitable levels along the digestive tract. Although in the present study, ingesta could repeatedly be drawn off from such regions as the rumen, abomasum, duodenum, ileum and caecum, attention has up till now been focussed mainly on some aspects of the large number of physiological processes occurring within the rumen.

Repeated collection of ingesta from the rumen (photo No. 9) is easily carried out by the insertion of a glass tube through the fistula opening into the depth of the ruminal mass and aspirating by mouth the required amount of ingesta into a splash-head bulb situated in the centre of the sampling tube. Photo No. 10 shows the collection of ingesta from the abomasum.

Photo No. 9.—Collection of ruminal ingesta by aspiration.

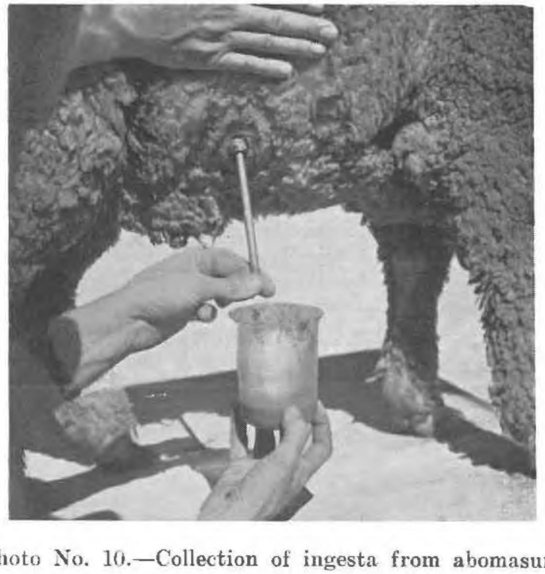

Photo No. 10.—Collection of ingesta from abomasum.

From the chemical aspect, ruminal contents have thus far been investigated in respect of moisture content variations, hydrogen ion concentrations and buffer system, the breakdown of different classes of carbohydrates including fibre, and the subsequent elaboration of organic acids both volatile and non-volatile.

Simultaneously with the above investigations, the material from the rumen has been subjected to close microscopic examination both of the bacterial flora and of the infusorial fauna, counts of which were made at repeatedly short intervals. No significant experimental work has, however, been undertaken thus far on the cultivation *in vitro* and the isolation of any organisms.

Significant differences, which will be discussed in subsequent publications, have already been noted in regard to both the chemical and microscopic aspects between animals existing on an adequate level of nutrition as compared to others on a deficient diet.

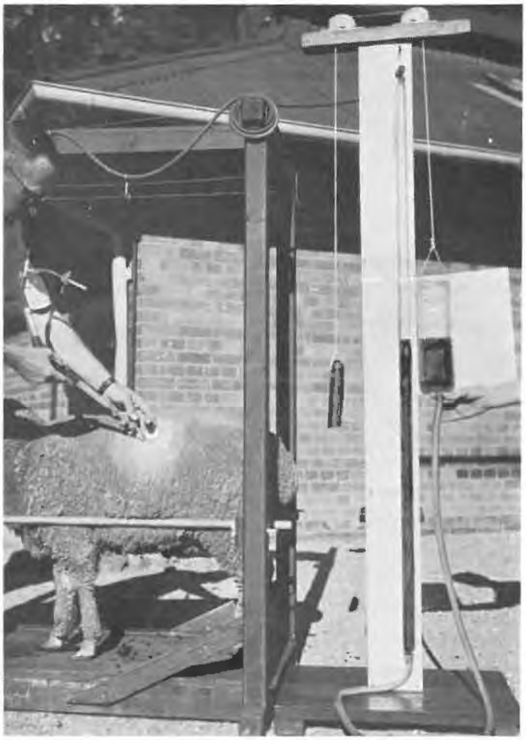

Photo No. 11.—Apparatus for recording total gas volumes from rumen at constant pressure. From side tube sampling of gas for analysis.

(c) *Sampling and Recording of Gas from Rumen.*

Seeing that the process of food disintegration within the rumen is associated primarily with fermentation, the continuous recording of the total gas volume liberated, together with an analysis of such gas, furnishes a significant index as to the rate and nature of the fermentation occurring at any given moment, and on a particular type of diet.

A convenient apparatus, devised for the continuous recording of rumen gas, is shown in photo No. 11. It is comprised of a manometer, one limb of which consists of a graduated tube 152 cm. long and 1,130 c.c. capacity, mounted on to an upright wooden stand. This tube is connected by means of a length of stout rubber tubing to a short cylindrical glass reservoir of wide bore, suspended from a pulley and capable of extensive up and down movement. The manometer is filled with the requisite amount of 1 per cent. HCl containing a little methyl red as indicator. The upper end of the graduated tube is connected through a length of rubber tubing directly on to the ruminal fistula in such a manner that any change of pressure within the rumen either depresses or elevates the column of fluid within the graduated tube. By continuous compensatory movements of the cylindrical reservoir, the pressure of the gas can be kept constant at atmospheric pressure. Continuous manometer readings are recorded over periods of 5 minutes at a time, each figure being plotted graphically as it is taken. In this way it is found that a biphasic pressure variation is frequently encountered in the rumen especially after a period of active fermentation, the pressure usually oscillating between a definitely positive and an equally definitely negative phase. This is obviously due to the fact that the pressure exerted by the gas within the rumen is determined not only by the speed and extent of gas formation itself, but largely also by the repeated changes in tonus of the musculature of the rumen. Hence a negative gas pressure is shown whenever the rumen passes into a phase of passive relaxation. By placing the zero line at a suitable level, both phases can be accurately recorded on the graph.

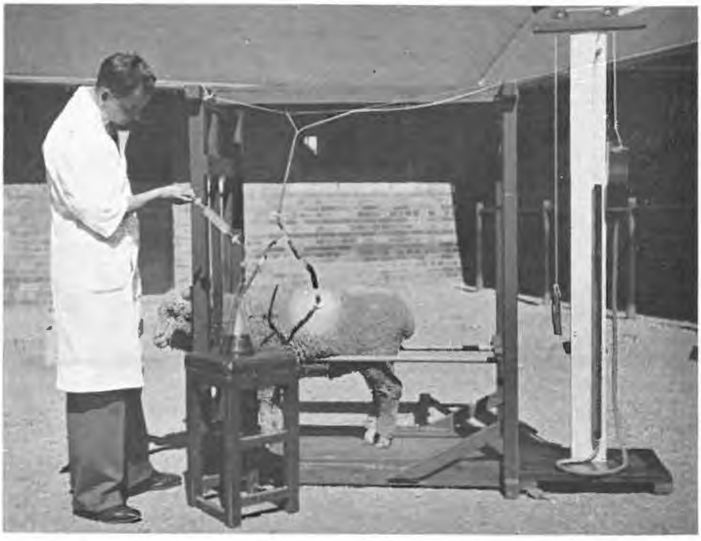

Photo No. 12.—Collection of gas from rumen for chemical analysis.

For the collection and analysis of gas samples, a T-piece with side tube and stopcock is introduced into the system close to the ruminal fistula. Samples of approximately 50 c.c. gas are collected in a small, specially fitted, glass cylinder by displacement of 1 per cent. HCl solution with which the cylinder is filled. (See photo No. 12.)

The gas is subsequently transferred into an evacuated gas pipette, and the analysis carried out in an Orsat-Lunge gas apparatus. The chief components of rumen gas is carbon dioxide which is determined by absorption in KOH solution and methane which is ignited electrically in the presence of oxygen. The percentage methane present is subsequently calculated from the amount of CO_2 formed from its combustion. (See photo No. 13.) Besides these two gases, smaller amounts of hydrogen, sulphuretted hydrogen, and nitric oxide may be encountered within the rumen. Seeing that the process in the forestomachs is essentially of the nature of a controlled acid fermentation, without much evidence of actual putrefactive changes, the formation of SH_2 and other evil-smelling gases is kept down to mere traces under normal conditions of digestion.

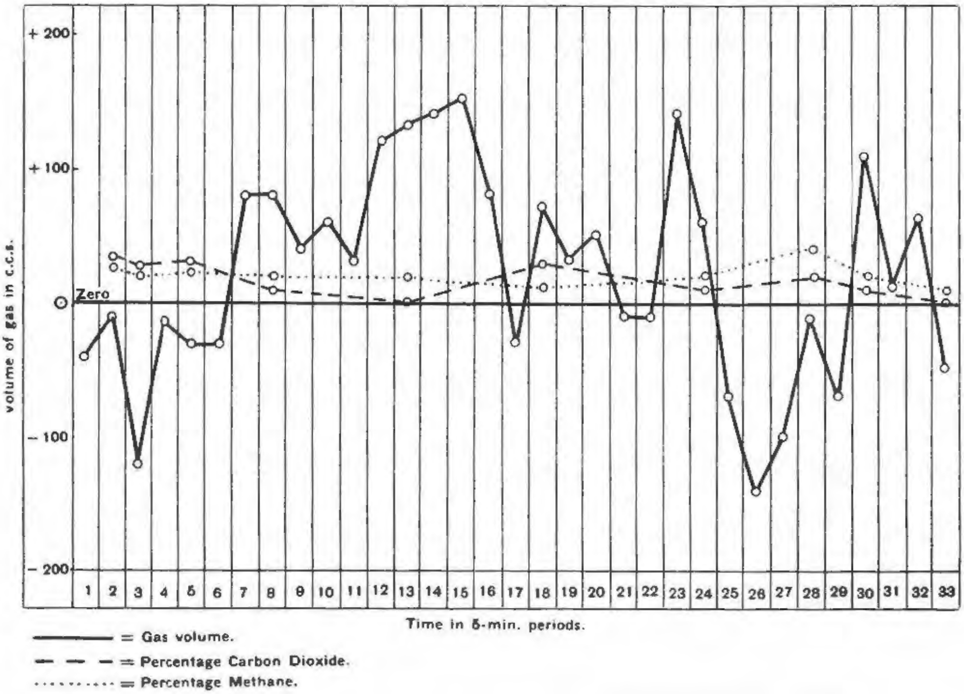

Photo No. 13.—Chart showing fluctuations in normal gas volumes.

(*d*) *Administration of Materials through Fistulae.*

Apart from allowing for the easy collection of contents, fistulae in the digestive tract offer another great advantage in that a wide variety of materials either in solid or in liquid form may be administered through such fistulae directly into any desired part of the tract. Such important factors as rate of passage, nature and speed of disintegration of a substance, disappearance through absorption, effect on digestive tract or animal as a whole, may all then be followed without much of the difficulties usually encountered in studies of this type.

So far, again, attention has been focussed mainly on the rumen. Weighed amounts of substances such as sugar or starch can be dosed through a glass funnel inserted into the fistula tube as shown in photo No. 14. The advantages of this method are that it obviates the necessity of first dissolving the material, frequently in large amounts of water for dosing *per os*, or through a tomach tube. Moreover, it ensures that all the material immediately enters the rumen, thus excluding the possibility of a portion being swallowed through into the abomasum. By these means, such factors as the rate and speed of fermentation of definite quantities of the different foodstuffs can easily be studied in animals existing on different diets, and hence significant data obtained in regard to both the normal and abnormal predigestion of food in ruminants.

Photo No. 14.—Administration of materials through fistula directly into rumen.

Similarly the process of disintegration of coarse materials within the rumen can be followed over an extended period of time. This is achieved by filling small cylindrical bags, made from a very thin natural silk, with samples of the particular foodstuff to be tested, such as dry grain (whole, crushed or shelled) or short lengths of grass or straw (see photo No. 15). By attaching a thin strand of silk to one end, the bag is then inserted through the fistula opening and kept suspended within the rumen by this strand of silk which is fixed to the outer part of the tube after closure of the external opening with its usual rubber cork. (See photo No. 16.) By repeatedly withdrawing the bag from the rumen, and gently washing it in running tap water, the contents of the bag can be examined either grossly or a more detailed microscopic study undertaken of the histological changes taking place within the different plant tissues. Subsequently the same material can be re-introduced into the rumen for a further period of disintegration. This method has also proved of value in comparing the activity of the ruminal flora with that encountered in other levels of the tract such as the caecum when the same materials are suspended through fistulae in the different organs.

Photo No. 15.—Filling of silk bags with various foodstuffs.

Photo No. 16.—Suspension of silk bags filled with foodstuffs within rumen.

Summary.

1. The importance is stressed of a more intensive study of digestion in ruminant animals, whose food, apart from being disintegrated by enzymes liberated from the digestive tract itself, is first of all subjected to a predigestion, the significance of which is as yet not fully recognised. Through the action of a varied and rich bacterial flora accompanied by large numbers of different species of infusoria, the food mass is exposed to a carefully controlled fermentation occurring within the forestomachs and similar in many respects to that occurring in silage fermentation.

2. In order to study the different stages in the process of digestion, the use of permanent bowel fistulae in experimental animals has been widely resorted to in the past, and an account is given of some of the experimental methods which have been used.

3. An account is given of our experimental technique which involves the use of suitable ebonite fistula tubes and the creation of permanent " closed " fistulae at different levels of the digestive tract of adult Merino sheep.

4. In this study attention has thus far been devoted mainly to some phenomena exhibited within the rumen of fistula sheep, kept on various controlled diets.

5. A method has been devised whereby any pressure change occurring within the rumen could be accurately recorded on slow moving kymographs and the limits of fluctuation in the motility of this organ registered under varying conditions.

6. The technique is described whereby samples of ingesta could repeatedly be collected from the rumen and subjected both to chemical and bacteriological study.

7. By the use of a large water manometer attached to the rumen fistula, continuous volume recording of gas from the rumen could be conducted under constant pressure. Likewise repeated sampling of gas for chemical analysis could simultaneously be undertaken.

8. Methods are described whereby different materials could be administered through the fistula opening directly into the rumen. By enclosing small samples of a variety of foodstuffs within cylindrical bags made from thin natural silk and subsequently suspending these within the food mass of the rumen, the rate at which disintegration took place as also its nature, could be followed microscopically.

Acknowledgment.

In conclusion we wish to express our thanks to Mr. H. B. Carter, University of Sydney, Australia, for the plan of the sheep crush pen used in the foregoing experiments. Likewise, we wish to thank Mr. T. Meyer for the photographs and Mr. C. G. Walker for the drawings and other illustrations, also Messrs. N. K. Gillham and R. J. Briel for valuable assistance in the execution of the experimental work.

LITERATURE.

ALVAREZ, W. C. (1928). The mechanics of the digestive tract. 2nd Ed. Paul B. Hoeber, Inc. New York.

BERGMAN, H. D., AND DUKES, H. H. (1925). Observations on certain diurnal phases of rumination. *Jnl. Amer. Vet. Med. Assn.*, Vol. 67, pp. 364-366.

BERGMAN, H. D., AND DUKES, H. H. (1926). An experimental study of the mechanism of regurgitation in rumination. *Jnl. Amer. Vet. Med. Assn.*, Vol. 69, pp. 600-612.

BESSELAAR, H. J., AND QUIN, J. I. (1935). Studies on the alimentary tract of sheep in South Africa III. The influence of bowel anastomosis on the general nutritional state of Merino sheep. *Onderstepoort Jnl. Vet. Sci. and An Ind.*, Vol. 5, No. 2, pp. 501-514.

BRUGGEMANN, J. (1935). Über eine Methode zur Registrierung der Magenbewegungen beim kleinen Wiederkäuer. *Arch. Tierheilk.*, Vol. 69, pp. 296-298.

COLUMBUS, A. (1934). Der quantitative Verlauf der Entleerung des Pansens bei Schafen und Ziegen mit Berucksichtigung der Gesamtentleerung des Magen-Darmkanals. Thesis: Handelsdruckerei G.M.B.H., Wiesbaden.

CZEPA, A., AND STIGLER, R. (1929). Der Verdauungstrakt des Wiederkäuers im Röntgenbild. Urban und Schwarzenberg. Berlin u. Wien.

DUKES, H. H. (1935). Physiology of the ruminant stomach. *Lederle Vet. Bull.*, Vol. 4, No. 6, pp. 1-6.

DUKES, H. H. (1937). The Physiology of domestic animals. 4th Ed. Comstock Publishing Co., Ithaca, New York.

DUKES, H. H., AND SAMPSON, J. (1937). Gastrointestinal motility in the ruminant. *Cornell Vet.*, Vol. 27, No. 2, pp. 139-149.

KRZYWANEK, F. W. (1932). Das Wiederkauer. *Handbuch der norm. u. pathol. Physiologie*, Vol. 18, pp. 36-44.

KRZYWANEK, F. W., AND QUAST, P. (1936). Die Bewegungen des Pansens und Labmagen beim Schaf und ihre Beziehungen zueinander und zum Wiederkauen. *Pflüg. Arch. Ges. Physiol.*, Vol. 238, No. 3, pp. 333-340.

LENKEIT, W., AND COLUMBUS, A. (1934). Zur Prüfung des Schlundrinnen reflexes. *Arch. Wiss. Prakt. Tierh.*, Vol. 68, No. 2, pp. 126-133.

MAGEE, H. E. (1932). Observations on digestion in the ruminant. *Jnl. Exp. Biol.*, Vol. 9, p. 409-426.

MANGOLD, E. (1929). Handbuch der Ernährung und des Stoffwechsels der Landwirtschaftlichen Nutztiere. Vol. 2. Verlag Julius Springer, Berlin.

MANGOLD, E. (1935). Die Verdaulichkeit der Futtermittel in ihrer Abhängigkeit von verschiedenen Einflüssen. "Der Forschungsdienst." Sonderheft 2, pp. 35-48.

MANGOLD, E., AND KLEIN, W. (1927). Bewegungen und Innervation des Wiederkaüermagens. Georg. Thieme, Leipzig.

MÖNNIG, H. O., AND QUIN, J. I. (1933). Studies on the alimentary tract of merino sheep in South Africa I. Investigations into the physiology of deglutition. *Onderstepoort Jnl. Vet. Sc. and An. Ind.*, Vol. 1, No. 1, pp. 117-133.

MÖNNIG, H. O., AND QUIN, J. I. (1935). Studies on the alimentary tract of merino sheep in South Africa. II. Investigations into the physiology of deglutition. *Onderstepoort Jnl. Vet. Sc. and An. Ind.*, Vol. 5, No. 2, pp. 485-499.

SCHALK, A. F., AND AMADON, R. S. (1928). The physiology of the ruminant stomach (ovine). Study of the dynamic factors. *North Dakota Agr. Expt. Sta. Bull.*, No 216, pp. 1-64.

SCHEUNERT, A. (1927). "Das Wiederkauer." *Handb. Norm. u. Pathol. Physiol.*

TRAUTMANN, A., AND SCHMITT, J. (1933). Beiträge zur Physiologie des Wiederkaüermagens. III. Über den Schlundrinnen reflex bei kleinen Wiederkaüer. *Arch. Tierernahr. u. Tierzucht.*, Vol. 9, No. 1, pp. 1-10.

TRAUTMANN, A. (1933). Beiträge zur Physiologie des Wiederkäuermagens. V. Die Bewegungsformen des Pansens und der Haube beim Säugenden Wiederkaüer. *Arch. Tierernähr. u. Tierzucht.*, Vol. 9, No. 1, pp. 19-30.

WESTER, J. (1926). Die Physiologie und Pathologie der Vormägen beim Rinde. Berlin, Richard Schoetz.

WOODMAN, H. E. (1930). The rôle of cellulose in nutrition. *Biol. Rev.*, Vol. 5, No. 4, pp. 273-295.