Development and Use of the Esophageal Fistula: A Review

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One of the foremost problems in range and pasture nutrition is making an accurate assessment of the chemical and botanical composition of the diet of grazing livestock. In recent years the esophageal fistula has been used to obtain samples of the forage grazed by ruminants. These studies have been conducted under a wide variety of conditions in at least six countries on four continents.

The purpose of this article is to review the development and use of the esophageal fistula and to discuss present problems and practices. The medical literature is replete with references to human and animal esophageal fistulas due to congenital abnormalities, disease, and accidental injury. However, this article is restricted to experimental studies with domestic animals and reviews the literature through mid-1963.

Historical Development

The esophageal fistula technique is not new. It was reported early by the famous French physiologist, Claude Bernard (1855). In fact, it was used by his teacher, Magendie, several years earlier in the horse (Magendie and Ryer, 1847). Pavlov's classic studies involving esophageal-fistulated dogs were initiated in 1889 (Pavlov, 1897). Some of his dogs lived for many years on food fed directly into the stomach. However, it is only in the last decade that the technique has been used widely in ruminants. A review of some early uses of the esophageal fistula in various species is outlined in Table 1. Most studies have been physiological or psychological rather than nutritional in nature.

[1] *The following are acknowledged for their review or suggestions: Drs. G. P. Lofgreen, J. H. Meyer, and W. C. Weir (California), C. W. Cook (Utah), and V. R. Bohman (Nevada).*

Surgical Techniques

At best, surgery of the esophagus is difficult; this accounts for numerous losses and for skepticism regarding the technique. Saint (1929) pointed out the following as major reasons why surgery of the esophagus is difficult: (1) the esophagus lacks a serosa, (2) in order to expose the esophagus in surgery it is necessary to open the mediastinal structures and fascial planes, (3) there is a very poor blood supply to the esophagus, (4) the esophagus cannot be restricted from movement, (5) there is an absence of the greater omentum. Further details concerning esophageal surgery are given by Saint and Mann (1929).

Early physiologists often considered it necessary to accomplish fistulation in a two-step operation because of these difficulties (Markowitz, 1954). Dragstedt and Mullenix (1931) reported over 50 percent mortality in making a one-stage fistula of the esophagus in dogs. In the two-stage operation the esophagus is first exteriorized in the neck; later, the esophagus may be sectioned without danger of mediastinitis. A two-stage procedure recently has been used in ruminants in establishing saliva collection cannulae (Whitmore *et al.*, 1963 unpublished manuscript).

Descriptions of surgical techniques for large animals have been published by Torell (1954), Cook *et al.* (1958), Hamilton *et al.* (1960), McManus (1960)[2], McManus *et al.* (1962b), Chapman and Hamilton (1962) and Cook *et al.* (1963). The following is a brief description of an un-

[2] *McManus, W. R. 1960. The development and use of oesophageal fistulae in sheep. Ph.D. Diss., U. New South Wales (Australia) 338 pp.*

Table 1. Early uses of the esophageal fistula in domestic animals.

Worker (s)	Year	Species
Bernard	1855	Horse
Colin	1856	Horse
Lobasov	1896	Dog
Pavlov	1897	Dog
Best and Cohnheim	1910	Dog
Nikovlina	1919	Geese
Karpov	1919	Geese
Collip	1922	Hens
Haberland	1926	Dog
Komarov	1926	Dog
Dragstedt and Mullenix	1931	Dog
Wilder and Stokes	1931	Pig
Bellows and Van Wagenen	1938	Dog
Heyenga	1938	Pig
Adolph	1939	Dog
Bellows	1939	Dog
Goldman	1939	Cattle
Wise *et al.*	1940	Calf
Janowitz and Grossman	1949	Dog
Torell	1954	Sheep
Mook	1962	Rat

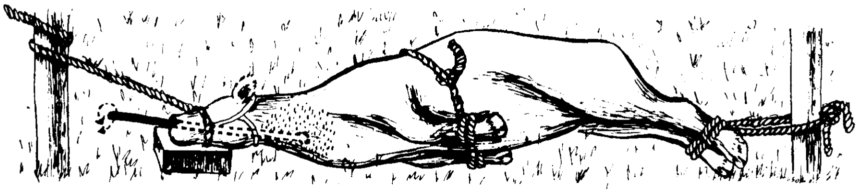

FIGURE 1. An animal positioned and restrained for esophageal fistulation. The stipuled area is clipped and disinfected. A steel rod with a hard rubber ball on the end is passed down the esophagus and used to position the esophagus in surgery.

published technique used successfully in both cattle and sheep[3]. The animal is adjusted to pelleted feed or green herbage for several weeks prior to surgery, but feed and water are withheld for 24 hours immediately prior to surgery. After the animal is anesthesized "to effect" by means of a general anesthetic, it is placed in a right lateral recumbancy and the rear legs are extended and tied (Figure 1). The forelegs are doubled back under the body and tied by means of a butterfly (or "tomfool") knot. The surgical area is disinfected and clipped. The head is slightly elevated and held by means of a halter rope. A steel rod with a hard rubber ball on the end is passed down the esophagus and manipulated to aid in making the incision and blunt dissection through the tissues (Figure 1). After the rod is passed, the supporting block (Figure 1) is moved back under the neck so that fluids will drain from the nose and mouth. The fistula should be located as near as possible to the ventral midline of the neck and about midway between the jaw and the brisket. After removal of an oval-shaped piece of skin (the size of the fistula), the tissues and muscles are separated and the esophagus is exposed. A short longitudinal incision is made in the esophagus and the sides of the incision are sutured. Sutures pass through the esophagus, submucosa, and inner layers of the skin. Incision and suturing is continued until the entire perimeter of the fistula is sutured. The cannula or plug is then inserted and the area disinfected and treated with fly repellant. The animal is kept off coarse feed and water for 24 hours postoperative, or is returned to grass. Antibiotics are given until the incision is healed. The sutures may be removed in seven to ten days. An experienced surgeon and two assistants can complete a fistula in a steer in about one hour.

McManus (1962b) suggested putting animals on green grass as soon as possible after surgery. Cook *et al.* (1958) recommended continuation of pellet feeding. However, care must be taken to prevent fistulated animals being fed pellets from consuming straw bedding or wood shavings which may become lodged and compacted in the esophagus (Goldman, 1939; Van Dyne, 1960). Tribe and Peel (1963) allow lambs to resume grazing and nursing immediately after surgery.

Fistulation Success

Success with large animals varies widely. In early developmental stages losses were great. Torell (1954) first reported success in only one out of four sheep. Later, he established an esophageal fistula in a heifer but she died of a digestive disorder before use. Cook *et al.* (1958) reported on fistulation of four sheep, all of which survived surgery. However, one lost its cannula due to necrosis within two months after installation. Four other sheep were fistulated but did not survive for various reasons. Lesperance (1959)[4] fistulated four steers, but none of these animals survived a year and some survived only a few weeks. McManus (1962a, 1962b) reported on esophageal fistulation of 35 sheep; he found 14 percent suitable for field studies. Some sheep were suitable for pen studies although not for field use. More than half of the fistulated sheep were not suitable for either pen or field studies. Nelson (1962) established stainless steel cannulae in four steers, but all were unsatisfactory over any extended period. He lists the following complications: 1) loss of cannula due to pressure necrosis; 2) recurrent lack of appetite; and 3) ulceration of the rumen and reticulum.

Recently, greater success due to development of more efficient closure devices has been reported. Van Dyne (1962) used five steers and seven sheep fistulated by the techniques described above. All the steers survived surgery and field use; all sheep survived surgery, but two were killed after several months use. Torell and Bredon (1961) established fistulas in 18 Ankole and Zebu cattle which survived a six-month study in good condition and at last report were still usable. Cook *et al.* (1961, 1962) have reported on use of fistulated sheep under range conditions. Several of their sheep have been used in more than one season with some now collecting in their fifth year. Wethers and calves have been fistulated successfully at the Grassland Research Institute in England (Lambourne, 1963, and personal correspondence), lambs and wethers in Australian and New Zealand studies (McManus *et al.* 1962b; Tribe and Peel, 1963; Ar-

[3] *A detailed outline of surgical procedure and care of animals is available on request.*

[4] *Lesperance, A. L. 1959. The development of techniques of evaluating grazed forage. M. S. Thesis., U. Nevada, 74 pp.*

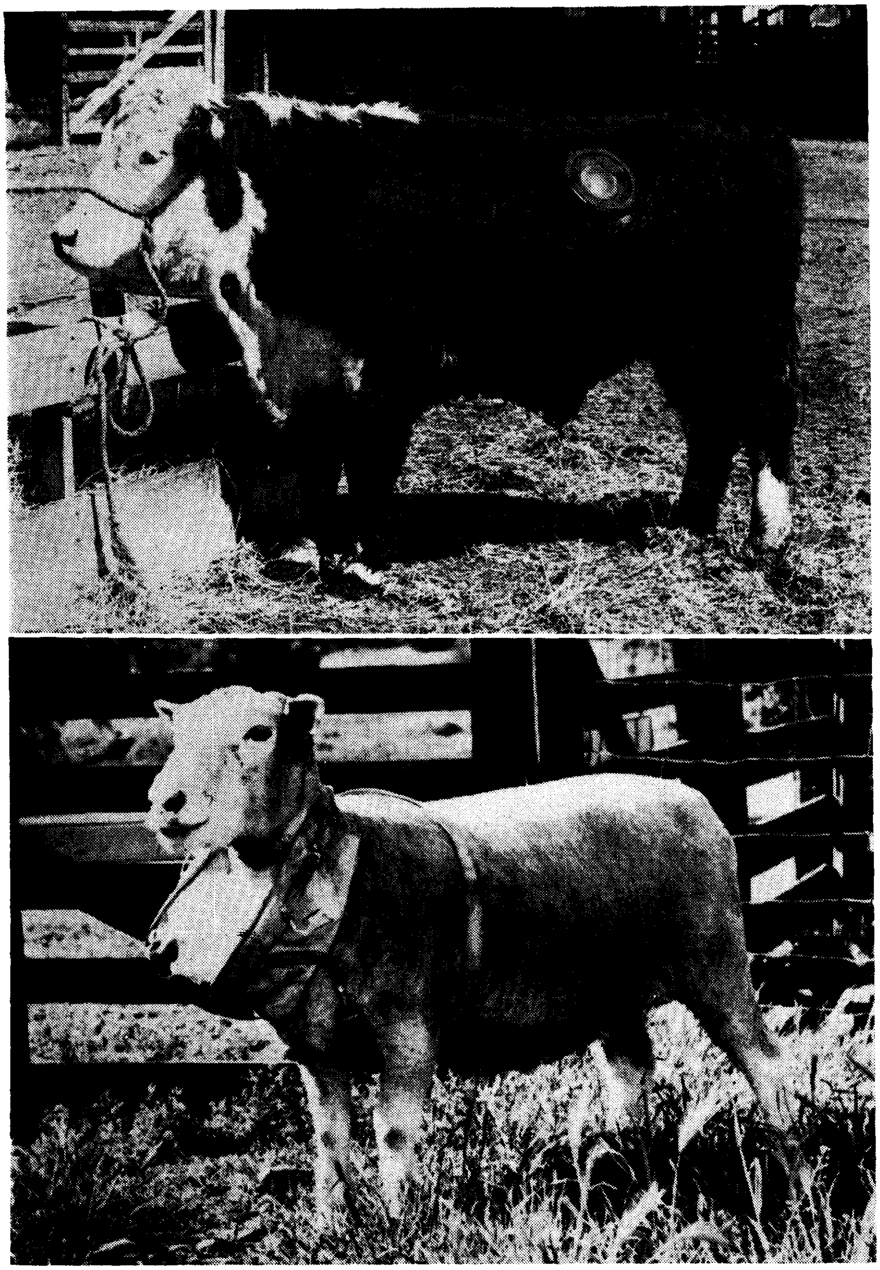

FIGURE 2. Examples of long term esophageal fistulated animals: The esophageal fistula was installed in the steer over two and a half years prior to the photograph and the rumen fistula for about two years; the ewe was fistulated in 1960 and has since been used in grazing studies.

nold *et al.* 1963; and W. H. Bishop, personal correspondence), and dairy cattle in the southern United States (G. H. Rollins and L. L. Rusoff, personal correspondence).

Greater surgical success has been obtained with young animals than with mature animals and fistulation is probably more successful in cattle than in sheep. Still, probably about ten percent of the animals fistulated by current techniques will not be useful over a long period of time, due to various operative and post-operative losses. Yet, some animals are serviceable for several years. Heady and Torell (1959) reported on an esophageal-fistulated wether which had been in use four years at the time of their study. Rusoff and Foote (1961b) reported using esophageal-fistulated dairy cows for two years. The bifistulated steer in Figure 2 has both an esophageal and a ruminal fistula. The esophageal fistula had been established about 2½ years at the time of the photograph, and the ruminal fistula for about two years. Fistulated ewes which raised lambs have been used (Van Dyne, unpublished data; Arnold *et al.,* 1963).

Types of Closure Devices

Various devices have been used for closing the esophageal fistula. Torell (1954) described the use of two stainless steel pins inserted into imbedded polyethylene tubing. The exposed end of the pins was held together by a cord or rubber bands. More recent closure devices are schematically illustrated in Figure 3. Type A represents a device used by Torell (1954) and by Van Dyne (unpublished). Two plastic plates are drawn together by nylon cord. Usually, a piece of cork or foam rubber is placed between the two plates of this completely removable plug. Lesperance (1959)[4] illustrates a similar device wherein the inner plate was wired to a metal outer plate. The outer plate was held in place by a strap around the animal's neck.

The nonremovable type of cannula (Figure 3B) has been used by various workers; it is constructed of lucite or acrylic plastic or stainless steel (Cook *et al.,* 1958; Van Dyne and Van Horn, 1959; Lesperance *et al.,* 1959, 1960a; Rusoff and Foote, 1961a, b; and others). The cannula in Figure 3D (Van Dyne, unpublished) has three important advantages over that in Figure 3B: 1) less esophagus need be incised to install the cannula; 2) it can be removed completely if necessary; and 3) when the plug is in place, the plate of the cannula does not have a hole in which forage may become lodged.

The plug shown in Figure 3C

has been in use for several years under a wide variety of conditions (Van Dyne, 1962; Torell and Bredon, 1961; and Tribe and Peel, 1963). Various size plugs can be interchanged to accommodate changes in the fistula. In use, both closure devices C and D are made so that the plug portion is "off center." This allows periodic switching of the long and short ends of the plate and aids in mainteance of a healthy fistula. Often there is a tendency for a "pouch" to pull down anterior to the fistula; periodic switching prevents this.

The molded latex plug in Figure 3E is one of several types described by McManus *et al.* (1962b). They also describe split plug stoppers made from surgical rubber. One disadvantage of this type plug under range conditions is that it pulls out relatively easily when caught in fences, brush, or in the animal's rear hooves when scratching. Nelson (1962) has used two "L-shaped" pieces of plastic held in place by two bolts for a closure device (Figure 3F). Spacers can be used in such a device to adjust its length. Wise *et al.* (1940) described a double-opening fistula in a dairy calf which was fitted with a rubber tube inserted into the exposed ends of the esophagus to serve as a conduit for normal milk feeding. The type of closure device is not well described in many early experiments. Many of the early investigations were probably acute studies.

A removable cannula has some advantages over a permanently fixed cannula. The permanently fixed lucite or stainless steel cannula eventually may cause the development of a pocket or blind pouch anterior to the fistula and eventually may be expelled. All the plugs or cannulae in Figure 3 can be removed, interchanged, and modified except B. The lucite cannula has some advantage in cold-weather sampling because it can be used while the operator is wearing gloves.

Inside openings in cannulae are usually about three cm in diameter in sheep and four cm or more in cattle. Openings smaller than this may permit plugging of the cannula and compaction of the feed within the esophagus, or may limit the percent of forage collected. The size of the opening is important because the size of bolus varies with the type of feed eaten (Bailey, 1961).

Removable cannulae are placed in fistulae which may vary considerably in length and width. In the author's studies the cattle fistulae are oval and four to five cm long and about three cm wide. The sheep fistulae are correspondingly smaller. It is desirable to establish a uniform size and shape of cannula or plug which may be interchanged among animals, thus eliminating the necessity of maintaining individual animal equipment.

Collection Apparatus

Sample collection apparatus include plastic bags (McManus,

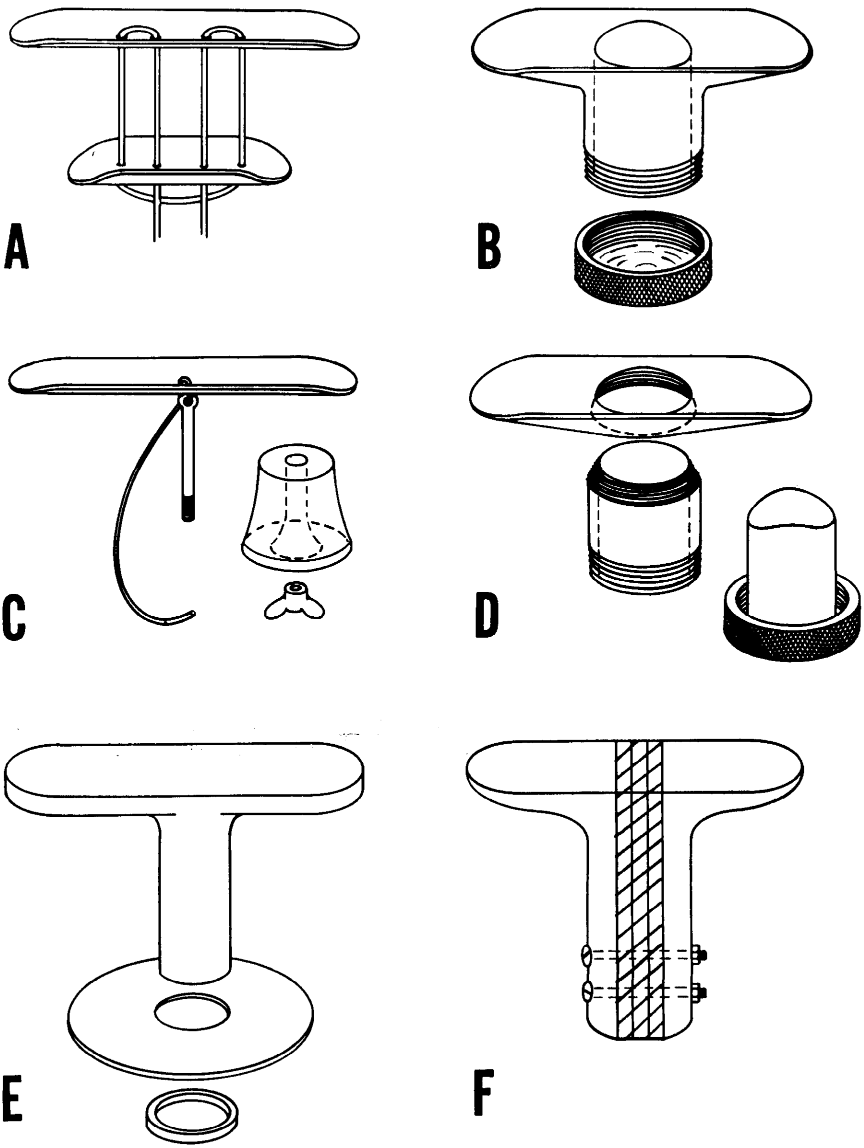

FIGURE 3. Schematic illustration of various types of cannulae and plugs used in esophageal fistulae in cattle and sheep (see text).

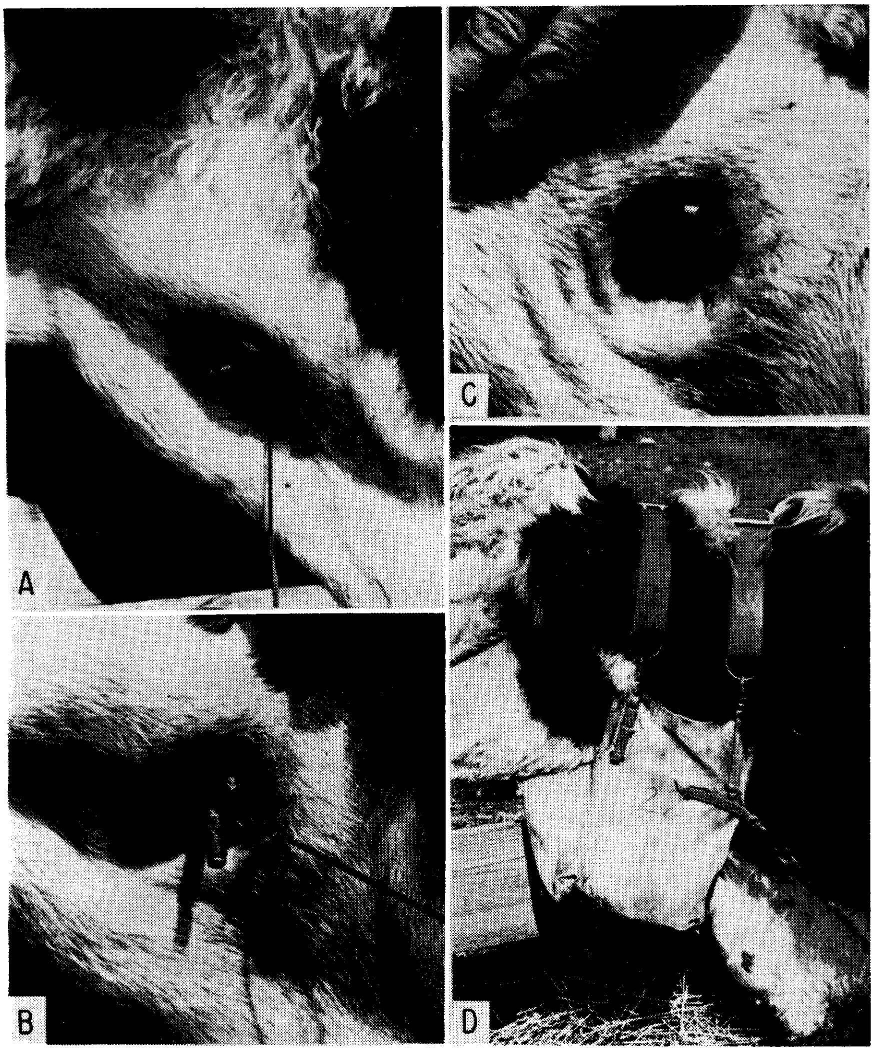

FIGURE 4. Close-up views of esophageal fistula: A. Plug in place; B. Rubber stopper portion of plug removed with plate and bolt still in place (see Figure 3C). C. Appearance of fistula with plug completely removed; and D. Screen bottom collection bag used on cattle (see also Figure 2 for sheep forage collection bag).

1962a; Lusk *et al.,* 1961), canvas bags (Torell, 1954; Cook *et al.,* 1958; Cook *et al.,* 1961), rubberized canvas (Lesperance, 1959)[4] and screen bottom bags (Van Dyne and Van Horn, 1959). The plastic bag without canvas protection is not suitable for most range investigations. The waterproof canvas bag can be a disadvantage under range conditions because a considerable weight of saliva and forage may accumulate and thus affect the grazing performance of the animal. The screen bottom bag allows saliva to drip off the sample (see Figure 4D). Thus, samples collected with screen bottom bags are less affected by salivary contamination than samples collected in plastic bags. This would be disadvantageous if nutrients were leached from the sample by saliva. However, McManus (1961b) has demonstrated by *in vitro* studies there is no significant leaching of nitrogen from succulent or roughage plant material.

The type of collection bag in Figures 4D and 2B can be rapidly attached to either cattle or sheep. Two adjustable straps with "D" rings are snapped over the neck. A small snap on the front of the bag attaches to the back of the halter. A strap on the back of the bag passes along the brisket and between the forelegs, through a "D" ring in a surcingle, and returns to snap to the bag on the other side. These bags are not displaced whether full or empty or whether the animal is browsing on high shrubs or grazing on low grass. A wire screen bottom is preferable to a nylon screen one because it holds the bag open and does not rip as easily in brush. Similar sheep collection bags may not require the straps at the front and back of the bag because the wool prevents slippage (see Cook *et al.,* 1961).

Length of Sampling

Sample volumes from one pint to one quart for sheep and half to one gallon for cattle are collected easily. McManus (1960)[2] reported 86 per cent of his sheep samples were $\geqq$ 20 g dry matter. Collection time depends upon the species, size of the fistula, rate of grazing, and type of forage. Bath *et al.* (1956) suggested a 30-minute maximum for collecting samples on irrigated pasture. Cook *et al.* (1958) reported two to four hours were required to collect an adequate sample under winter desert range forage conditions. Two-hour collection periods either in morning or evening grazing were adequate for sampling open foothill winter range (Van Dyne and Van Horn, 1959). Van Dyne (1963), in studying grazing of esophageal-fistulated steers and wethers on a common dry annual foothill range, used collection times of 1.4 to 2.4 hours. Sampling duration as short as ten to 15 minutes has been reported in forage studies with esophageal-fistulated sheep on small fenced plots (Lusk *et al.,* 1961) and with steers fed roughages or concentrates (Nelson, 1962). Collection periods up to one-hour duration resulted in 30-200 g samples from sheep (Arnold *et al.,* 1963).

McManus (1962a) infers from

his data that probably collection time should not exceed one hour in order to maintain normal conditions in the rumen. Extended loss of saliva may cause changes in digestive activity by allowing the accumulation of relatively large concentrations of volatile fatty acids in the rumen. If this accumulation leads to abnormally low pH levels, ruminal stasis could result. Whether this occurs under extended range sampling has not been demonstrated.

Chemical Analyses

Forage samples taken from esophageal-fistulated animals have been subjected to a wide variety of analyses. Various workers have analyzed samples for the proximate components—crude protein, ether extract, total ash, crude fiber, and nitrogen free extract (by difference). Other commonly determined constituents are lignin, cellulose, other carbohydrates (by difference), silica, phosphorus, calcium, and gross energy. Potassium (Rusoff and Foote, 1961b) and plant chromogens (Van Dyne, unpublished, 1959) also have been determined. Renet coagulation time, surface and body curd tension, pH, and lipolytic activity have been determined in milk samples from a sham-fed esophageal-fistulated calf (Wise *et al.*, 1940). No special changes in laboratory procedures have been reported for analyzing esophageal fistula forage samples. However, salivary contamination has been considered in some investigations (see next section).

MacDougall and DeLong (1942) and Van Soest (1962) have reported on effect of heat drying on lignin content in forages and in cattle and sheep feces. This heat drying effect may be important in drying fistula forage samples. Bohman (1958) found hay samples collected by the rumen evacuation technique had greater lignin content than the hay had before feeding. However, Sharp (1962)[5] found crude fiber in the forage sample to be greater than crude fiber in the rumen-collected sample. Ensalivation of the sample and high-temperature drying may bias lignin values. MacDougall's and DeLong's (1942) and Van Soest's work (1962) show water and high drying temperature cause a nonenzymatic browning reaction in which carbohydrate degradation products condense with protein. This dark colored polymeric material is insoluble in 72 percent H_2SO_4 and thus would cause a positive bias in the lignin determination. Recent work has indicated this material may be modified xylans (Gaillard, 1962). Therefore, it may be desirable to keep the forage sample as dry as possible by use of screen bottom collection bags and to dry the samples at relatively low temperatures. More work is needed to establish the importance of drying conditions on lignin determinations in fistula forage samples.

Salivary Contamination

Fistula forage samples contain differential amounts of salivary contaminants, depending upon the type of collection bag. Bath *et al.* (1956) indicated salivary contamination increased the ash content of the sample while not appreciably affecting other constituents. McDougall (1948) reported sheep saliva contains about 0.8 percent ash, Lesperance *et al.* (1960a) found bovine saliva to contain 0.85 percent ash, and Bailey and Balch (1961) found bovine saliva dry matter and ash to be respectively 1.02 percent and 0.89 percent. The calculations of Lesperance *et al.* (1960a) indicate that ash, phosphorus, or calcium contamination from saliva would increase significantly the content of those components in the forage sample. They considered it doubtful that regression equations could be established to relate salivary contamination and feed composition when comparing samples from stall-fed animals and samples from animals grazing on pasture. Cook *et al.* (1961, 1962), however, have attempted to correct fistula forage sample composition for added nitrogen, ash, and phosphorus from salivary contamination. This procedure is as follows (C. Wayne Cook, 1963, personal correspondence): simulate grazing by hand collecting samples, determine moisture in these; collect saliva samples and analyze for moisture and chemical constituents; subtract plant moisture from the total moisture in the fistula sample to determine amount of salivary contamination; deduct amount of various constituents added by the saliva. However, this procedure is subject to criticism because of the assumptions: 1) that forage grazed can be sampled adequately by hand; 2) that saliva composition or secretion rate is invariable; and 3) that the fistula samples are completely saturated with either plant or salivary moisture, or both (screen bottom collection bags are used).

McManus (1961b) reported on collection of roughage feedstuffs from small fistulas and found recoveries generally exceeded 35 percent of the material fed. He stated that chemical composition of the extruded material was similar to that fed except for an increase in ash content. He suggested that results always should be expressed on an ash-free basis and similar results were obtained by Nelson (1962). Because trampled forage or seeds picked from the ground may

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[5] *Sharp, G. D. 1962. An evaluation of the rumen clearance technique for measuring the nutritive value of forage consumption under range conditions. M. S. Thesis, U. Idaho. 38 pp.*

have considerable soil contamination (Van Dyne, 1963), it may be desirable to present data on a silica-free basis. Probably a silica-free basis or an ash-free basis is the desirable way to present data unless an accurate procedure is available with which to correct for salivary contamination.

Isotope-dilution Technique

It is possible, by use of isotope-dilution procedures, to measure the amount of moisture or various mineral constituents added to fistula forage samples by the saliva. The isotope-dilution procedure is relatively simple. The animal is "dosed" with the isotope and allowed to come to an equilibrium, saliva samples are taken before and after forage collection, and saliva and forage are analyzed chemically and for radioactivity. The total amount of a given mineral in the fistula sample (determined by chemical means) less the amount added by salivary contamination (determined by radioactivity analysis) is the amount in the forage. Luick *et al.* (1959) have described a method for determination of water intake of grazing sheep by using tritium. However, only limited pasture or range trials have been reported wherein forage moisture content has been evaluated by isotope-dilution techniques (Torell, 1958).

It is necessary, however, to analyze accurately the saliva before and after collection of the forage sample. Bailey and Balch (1961) and Somers (1961) have shown that the amount and composition of saliva can vary considerably, depending upon the type of forage consumed. This precludes accurate estimates of salivary contamination by use of prediction equations based on controlled feeding of roughages considerably different in physical nature from grazed forage.

Esophageal vs. Ruminal Fistula Sampling

Esophageal fistula and ruminal fistula sampling were compared by Lesperance *et al.* (1960a). When the animals were fed alfalfa hay, the esophageal fistula samples usually contained more nitrogen-free extract than did the ruminal fistula samples. Samples from both esophageal and ruminal fistulas were considerably different in composition from the feeds fed. The importance of selective consumption even by stall-fed cattle recently has been emphasized by Bredon and Marshall (1962). They found that cattle were able to select certain parts of the roughages fed and that portions eaten were superior to the average of the feed offered. Sharp (1962)[5] measured the amount and composition of the feed refused and subtracted that from the feed offered to study the composition of samples obtained through ruminal fistulas. His data showed fewer significant differences for various nutrients, between forage fed and ruminal fistula samples, when the results were expressed on an organic matter basis rather than on a dry matter basis. His differences between herbage and forage composition may be related to the nonenzymatic browning reaction mentioned above.

To sample range or pasture with the rumen-evacuation technique involves 1) completely emptying the animal's rumen, 2) allowing the animal to graze, 3) taking the forage sample from the rumen, and 4) replacing the rumen contents. The rumen-evacuation technique is not suitable for repeated sampling under range conditions. The technique is more time consuming than that of the esophageal fistula and presents some obvious disadvantages on cold, open winter range. It is also difficult to make direct comparisons between cattle and sheep by the rumen-clearance method. It has been shown that emptying the rumen even as few times as thrice weekly has a depressing effect upon digestibility of forage (Lesperance and Bohman, 1963). Thus, repeated sampling (e.g. morning and evening grazing on several consecutive days) could have a considerable effect on the animal, and subsequently on his grazing performance.

Tayler and Deriaz (1963) used a rumen-fistulated steer to collect pasture forage samples by the following procedure: "While the steer grazed, the collector's arm . . . was inserted through the orifice of the fistula into the rumen. Boluses of ingested herbage were collected in the palm of the hand as they reached the cardia." Such a procedure would be objectionable under range conditions because: 1) the free movement of the animal would be hampered, 2) few animals could be used, and 3) keeping the animal's rumen empty could alter selectivity.

Digestion of Fistula Samples

Salivary contaminants in the fistula forage samples do not invalidate the samples for microdigestion estimates. Bailey (1962) studied the rate of digestion of swallowed and unswallowed grass in nylon bags. The crushed and ensalivated grass samples had an increased rate of digestion up to about 13 hours. Beyond 13 hours there was no significant difference in digestibility of the swallowed and unswallowed grass. Most investigators use at least a 24- or 48-hour fermentation period for nylon bag and artificial rumen digestion of range and pasture forage samples (Kercher, 1962; Van Dyne, 1962; Tayler and Deriaz, 1963).

Botanical Analyses of Fistula Samples

Certain plant parts and individual plant species may be identified in both esophageal and ruminal fistula samples. Torell (1956) used a microscope to identify plants in hand-made, two- or three-species mixtures. He recorded the nearest plant under a crosshair of the scope with a stage with fixed stops. Six hundred points were recorded per sample. His data indicated percent points closely approximated percent weight for simple mixtures.

Cook *et al.* (1958) stated that browse plants in fistula forage samples could be almost completely identified on the basis of textural and color differences, whereas grasses and herbs from winter desert range frequently were masticated to the extent that they were too fine for visual identification. Heady and Torell (1959) studied forage preferences of esophageal-fistulated sheep by determining botanical composition by the microscopic point technique. Samples were washed, then spread on a five x 30-inch tray and passed under a microscope. Four hundred points were recorded for each sample. Their samples, collected from February through July, showed a wide variation in species composition and stages of maturity. Identification of species and plant part usually was possible, but required considerable training. Lusk *et al.* (1961) obtained plant parts from various species in the field prior to fistula collections to aid in identification under the microscope. They washed the fistula samples in 2 percent acetic acid to remove saliva before reading 200 points per sample. They only recorded hits as Medusa-head or other green forage. Lesperance *et al.* (1960b) placed their samples in Petri dishes and recorded a total of 100 points per sample. Only grasses and broad leaf plants were differentiated in their investigation. By similar techniques, Ridley *et al.* (1963) determined percent composition of four components in ruminal fistula forage samples. In recent investigations, Van Dyne (1963) has presented data wherein 200 microscopic points were read per sample by species or genus of fistula forage from steers and wethers grazing a common dry annual foothill range. An average of five to seven percent of the points were recorded on forage particles unidentifiable as to plant group (grass, forb, or shrub) but identifiable as to plant part (head, stem, or leaf). All samples contained at least a small amount of completely unidentifiable material. Recently, equations have been developed for prediction of percent weight from percent microscopic points. The correlation coefficients for many species and groups are from 0.92 to 0.96 (Heady and Van Dyne, 1963, unpublished). Colorado workers have made microscopic determinations on fistula forage samples ground through a Wiley Mill (F. C. Daugherty, 1963, personal correspondence).

Sampling Frequency

Several variations are apparent in sampling procedures (Table 2). Some investigators keep animals off feed overnight prior to collection periods (Bath *et al.*, 1956; Cook *et al.*, 1958; Weir and Torell, 1959). Keeping the animals off feed has an effect on their forage selectivity the following morning (Arnold *et al.*, 1963). However, other workers (Van Dyne and Van Horn, 1959; Van Dyne, 1960; Price, 1963, personal correspondence) allow the fistulated animals to run with the band or herd day and night. Small bands or herds of experimental animals were grazed together in other researches (Cook *et al.*, 1961, 1962; Van Dyne, 1962). Lesperance (1959)[4] placed fistulated steers on pasture only one day prior to sampling. Arnold *et al.* (1963) found this is undesirable with sheep. Further work is needed to evaluate the influences of fasting, herd size, and the animals' familiarity with the range.

Sampling frequency varies widely according to the purposes of the investigation (Table 2). Most investigators sample only once in a given day, but others sample twice daily, adjusting their sampling scheme to the normal grazing activity of the herds or bands involved (Van Dyne and Van Horn, 1959; Butcher and Cook, 1960; Van Dyne, 1962 and 1963). If the animals are hungry they are more likely to graze vigorously (Arnold *et al.*, 1963), and there is less chance for contamination of samples with regurgitated material. On hot summer range where grazing may be primarily during the morning and evening coolness, there is considerable opportunity for such contamination. However, on open winter range, or on cool summer range, regurgitation is not common. For example, in one study on summer mountain range, 90 fistula samples, averaging 82 gm of oven-dry weight, were collected from five esophageal-fistulated sheep during three periods. Only five of these samples were contaminated by regurgitation (Price, 1963, personal correspondence).

Samples were collected only once a month in some investigations (Weir and Torell, 1959). However, it is more common to collect samples on four or more successive days for estimating qualitative forage intake in digestion trials (Cook *et al.*, 1961, 1962; Van Dyne, 1962; Price, 1963, personal correspondence). In some investigations samples are collected for several months on alternate days, or every fourth day, to provide an estimate of seasonal changes in qualitative intake (Van Dyne and Van Horn, 1959; Van Dyne, 1960). Since significant differences can occur in the chemical

Table 2. Use of esophageal-fistulated animals in grazing studies.

Worker(s)	Year	Class of stock	No. of animals	Location	Type of grazing	Season	Sampling scheme
Bath, *et al.*	1956	Sheep	2	California	Irrigated pasture	Summer	Once daily, 3 times per week
Heady and Torell	1959	Sheep	3	California	Annual range	Spring-summer	Once daily, 1 day per period, pre-fasted
Torell and Weir	1959	Sheep	2-3	California	Annual range	Spring-summer	Once daily, 1 day per period, pre-fasted
Van Dyne and Van Horn	1959	Ewes	4-9	Montana	Foothill range	Winter	Twice daily, every fourth day, during 3 months, not fasted
Weir and Torell	1959	Sheep	6	California	Irrigated pasture Annual range	Summer Yearlong	Once daily, once per month, pre-fasted
Butcher and Cook	1960	Sheep	6	Utah	Desert shrub	Winter	Twice daily, 7 consecutive days per trial, pre-fasted
Edlefsen *et al.*	1960	Sheep	2-3	Utah	Desert shrub	Winter	Once daily, 6-8 consecutive days per trial, pre-fasted
Lesperance, *et al.*	1960b	Steers	1-2	Nevada	Irrigated pasture	Summer	Once daily, about every 2nd or 3rd day per trial
Van Dyne	1960	Heifers	4	Montana	Foothill range	Winter	Once daily, alternate days, for 3 months, not fasted
Cook *et al.*	1961	Ewes	4	Utah	Mountain range	Summer	Once daily, 5 successive days per period, pre-fasted
Lusk *et al.*	1961	Sheep	2	California	Annual range	Spring	Once daily, 1 day per period
Rusoff and Foote	1961b	Cows	2	Louisiana	Pasture	Summer	9-14 samples per trial (trial length unspecified)
Achacoso	1962	Cows	2	Louisiana	Pasture	Spring-summer	Compare fistula and hand clipped samples, several samples per trial
Cook *et al.*	1962	Wethers	3	Utah	Desert shrub	Winter	Once daily, 10 consecutive days
Nelson	1962	Cattle	4	Oklahoma	Bermuda grass	Summer	Twice daily, 4 consecutive days per trial
Van Dyne	1962	Sheep Steers	7 5	California	Dry annual	Summer	Twice daily, 5 consecutive days per period, not fasted
Arnold *et al.*	1963	Sheep	4-8	Australia	Annual range	Yearlong	Monthly or seasonal intervals, 3 consecutive days per period
Cook *et al.*	1963	Sheep Steers	8 2	Utah	Mountain range	Summer	Days unspecified, ½ animals for a.m. and p.m. sampling each, pre-fasted

composition of the diet from day to day even under relatively uniform conditions (Lesperance *et al.*, 1960b; Arnold *et al.*, 1963) it would appear desirable to have collections extending over several days or at least staggered over a time period. Yet, under band-grazing conditions, and sampling every fourth day both morning and night on dry winter foothill range in Montana, no significant differences were found between time of day, dates or animals. Five ewes were sampled twice daily on five days at four-day intervals (Van Dyne, 1959, unpublished data). Studies are now in progress to evaluate animal to animal variation as well as day to day variation on summer and winter range in various areas with cattle, sheep, or both. Results of these investigations should be useful in planning further sampling with esophageal fistulas (Butcher and Cook, 1960; Torell and Bredon, 1961; Van Dyne, 1963; Arnold *et al.*, 1963; Lambourne, 1963, personal correspondence).

Behavior of Fistulated Animals

It is difficult to evaluate whether the grazing behavior of the esophageal-fistulated animal differs quantitatively from that of the nonfistulated animal. Observations indicate that successfully fistulated animals graze normally (Arnold *et al.*, 1963). The fistulated animals graze among the others under herd or band grazing conditions. McManus (1960)[2] has harnessed esophageal-fistulated sheep for fecal collections. However, only a few of his sheep had "sufficiently successful" fistulas so that they could be used for both forage and fecal collections. He has indicated from other studies that the fistula must be established for three months prior to sampling for the esophageal-fistulated sheep to establish "gregaric-social relationships with their colleagues" and to become accustomed to their environment. Yet physiologically, the fistula is sufficiently well established in two to three weeks.

Esophageal-fistulated cattle can be halter-broken and gentled so that they may be caught on open range, thus greatly facilitating sampling. Esophageal-fistulated sheep intermixed in a large band of sheep may be bunched by a sheep dog and caught with shepherd's crooks; thus, special catch pens or corrals are not necessary (Van Dyne and Van Horn, 1959). These procedures allow handling of the fistulated animals under open range conditions with a minimum of disturbance to the whole band or herd.

Differences in grazing behavior of fistulated animals of different age and sex classes have not been reported. Also, it is not known if supplemental feeding alters feeding behavior and forage selectivity.

Areas Sampled

Esophageal-fistulated animals have been used successfully in a wide variation of environments varying from cold, open winter range in Montana and Utah to hot, dry summer range in California (Table 2). Fistulated animals have been used in groups of two to six in ten by 40-foot or 30 x 30-foot range plots or irrigated pastures (e.g. Bath *et al.*, 1956; Heady and Torell, 1959), and in small range paddocks of five to ten acres (e.g. Cook *et al.*, 1961, 1962, 1963); groups of four to nine head have been studied in bands or herds grazing on open range areas of several thousand acres (e.g. Van Dyne and Van Horn, 1959; Van Dyne, 1960). The same fistulated sheep have been used in a range band to follow seasonal intake as the band grazes on summer mountain range, spring-fall sagebrush-grass range, and winter salt desert shrub type range (Price, 1963, personal correspondence).

Accuracy of Samples

Accuracy of esophageal fistula sampling, defined as the closeness of the sample collected to the true sample grazed, is not known because true grazing samples are not known. However, in several investigations it has been shown that the samples obtained from esophageal-fistulated animals are considerably different from the gross herbage available (Weir and Torell, 1959). Samples taken by hand plucking or clipping while observing grazing animals are often considerably different in composition than the average herbage available (Hardison *et al.*, 1954; Edlefsen *et al.*, 1960). Probably, the fistula samples represent the best estimate available of the intake of grazing animals.

Lesperance *et al.* (1960a) have suggested that possibly large, coarse forages are not well sampled by a small esophageal fistula or by small non-removable cannulae in the same proportion to the material fed when compared to the rumen fistula technique. However, due to individual animal variation, this phenomenon is not definitely established. In laboratory trials with mixed diets containing wheat grain, wheaten chaff, and lucerne chaff, or diets with lucerne chaff alone, McManus (1961b) found no marked changes in the physical composition of the extrusa in sheep equipped with esophageal fistulas of from 1.5 to 3.0 cm diameter. There may be a class of stock x type of forage interaction when comparing esophageal and ruminal sampling techniques. There is perhaps greater opportunity for positive bias in collection of small plant particles, such as leaves, with permanently fixed cannulae than in sampling with esophageal fistulae with cannulae or plugs removed. The thin stainless steel cannula of Rusoff and Foote (1961a) has some advantage over the fixed acrylic or lucite cannulae because of its higher ratio of inside to outside stem diameter. A 3.8 cm outside diameter stainless steel cannula with 0.3 cm wall thickness was found suitable for use with grazing dairy cows. Still, the inside diameter of this cannula, about 3.2 cm, is considerably less than that of the 3.8 cm diameter plastic pipe cannula which Lesperance *et al.* (1960a) found unsatisfactory. The latter workers successfully used cannulae with an inside diameter of about five cm.

McManus (1960)[2] stated there was some indication that esophageal-fistulated sheep selected diets higher in nitrogen content than did intact sheep grazing in the same pasture. This was inferred from a comparison of the composition of the feces of the two groups. Differences were more apparent on perennial than on annual pastures. However, the fistulated sheep were placed on the pastures for sampling only intermittently, whereas the intact sheep remained on the pastures continuously. Also, there were breed differences between the two groups, thus confounding the measurements of intake. This problem, comparison of intact and fistulated animals, remains unsolved.

No exhaustive study has been made comparing effectiveness of esophageal and rumen fistulas for sampling a wide variety of green and dry range forages. This could best be tested in bifistulated cattle (e.g. Figure 2) because each animal could act as its own control and animal-to-animal variations would be minimized.

Precision of Fistula Samples

Weir and Torell (1959, unpublished) made multiple collections from esophageal-fistulated sheep grazing in small plots. As many as five collections, each 20 to 30 minutes in duration, were made from two of the sheep. Mean crude protein content of the diets of the two sheep varied from 19.8 to 25.2 percent; crude fiber varied from 13.9 to 15.5 percent. They concluded there was no advantage in making more than one collection per animal during a given period of the day. Similar results were found by Lesperance *et al.* (1960a). These data indicate that the technique may not be highly precise, but variation between animals is considerably greater than variation between samples for a given animal. Multiple collections need further evaluation under range conditions.

Other Uses

Tribe and Peel (1963) installed esophageal fistulas in lambs between four and 12 days of age for use in recording the resting secretion rates of total saliva in lambs. The rate of both total and parotid salivary secretion of lambs from 13 to 86 days of age and from two-year-old grazing wethers was recorded. The lamb fistulas did not enlarge as the lambs grew. The investigators report that at no time did their experimental animals show signs of unthriftiness, lack of appetite, or unusual behavior; weight gains were normal. They suggested utilizing esophageal-fistulated lambs to measure milk production in ewes.

The esophageal fistula technique is being used to answer various questions related to animal grazing behavior (Arnold, 1963, personal correspondence). Fistulated sheep have been deprived of various senses—sight, smell, taste, and touch—and fistula samples were taken to determine which senses animals use in making a choice of forage. Sheep from one area were moved to another area to determine their reaction to new plants and to new environmental situations with reference to forage selection.

Facial eczema caused by the fungus *Pithomyces chartarum* results in considerable loss to the sheep industry in New Zealand. Esophageal-fistulated sheep are used to collect ingested grass so that these spores can be counted (Bishop, 1963, personal correspondence). In similar fashion, Southcott (1962, personal correspondence) is collecting ingesta to determine the number of parasitic larvae consumed under various grazing management practices and anthelmintic treatments.

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