Acquisition and Analysis of Bovine Rumen Fluid

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Rumen fluid sampling has assumed increasing importance in veterinary practice in recent years. Descriptions of suitable sampling devices and of methods for analyzing the aspirated rumen fluid have appeared in the literature (Pounden 1954, Holtenius, Björck and Hoflund 1959) and have been included in textbooks of clinical methods for a fairly long time (Dirksen 1964, Dirksen 1977, Jaksch and Glawischnig 1976). However, the diagnostic and therapeutic possibilities afforded to the cattle practitioner by the acquisition of rumen fluid have not yet been dealt with to a satisfactory extent. Recently the collection and analysis of rumen fluid have acquired renewed importance through clarification of the pathogenesis and clinical signs of the abomasalruminal reflux syndrome. This and other currently recognized indications for collecting rumen fluid are summarized in Table 1.

TABLE 1. Indications for obtaining rumen fluid from cattle.

Diagnosis or exclusion of microbial dysfunction (indigestion).
Diagnosis of abomasalruminal reflux.
Chemical analysis for ingested toxins.
Evaluating the effects of new drugs on rumen microbial activity.
Emptying the rumen affected with overloading of fluid ingesta or with other dysfunction.
Obtaining rumen fluid from healthy cattle for therapy of forestomach disorders and metabolic diseases as well as for speeding the recovery from various systemic illnesses.

TABLE 2. Parameters that can be determined immediately after obtaining rumen fluid.

Color, odor, and consistency.
pH.
Methylene blue reduction.
Sedimentation (including protozoa) and flotation.
Total titratable acidity.
Chloride concentration.

TABLE 3. Important rumen fluid parameters that can be measured in the laboratory.

Glucose fermentation (gas formation).
Nitrite reduction.
Buffer capacity.
Concentration of volatile acids and lactic acid.
Ammonia concentration.
Protozoa (microscopic, quantitative).

Clinical evaluation of rumen fluid

Analyses that can be performed on the farm and in the laboratory are listed in Table 2 and Table 3. Rumen fluid normally has a not-unpleasant, aromatic *odor* and is somewhat viscous. The *color* is green or brown and depends on the diet (Figure 1). The fluid becomes watery and has a stale or indifferent odor when simple inactivity of flora and fauna is present, as with several days of inanition or when a high-fiber diet low in digestible nutrients is fed. A dark brown fluid with a repulsive fecal odor occurs with putrefaction of the rumen contents. A milky grey fluid with a sour odor (from weaned cattle) is typical of lactic acidosis. The sample is often dark brown with a sour odor reminiscent of bitter almonds when reflux of abomasal fluid into the rumen has occurred. The odor should be judged while the sample is still warm.

The normal *pH range* of rumen fluid is 5.5 to 7.0. Measurement of the pH with indicator paper[b] which shows gradations of 0.2-0.3 units is adequate for diagnostic purposes. The pH should be determined as rapidly as possible, as otherwise it may increase with loss of carbon dioxide or decrease with further fermentation. Values need

[a] *Guest at the 2nd Medical Animal Clinic, Munich University from August 1986 to July 1987.*

[b] *Special Indicator Paper 4.0-7.0 and 6.5-10.0, Merck, Darmstadt, West Germany.*

FIGURE 1. Sedimentation of feed particles and a grey layer of infusoria from active rumen fluid.

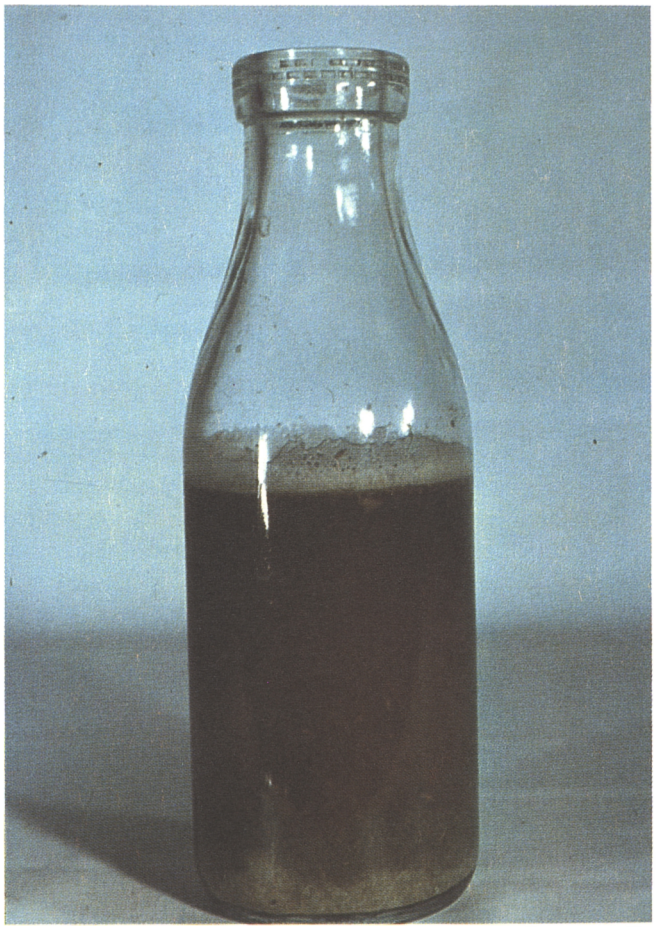


FIGURE 2. Effect of diet and time since feeding on rumen pH: ration rich in forage (-——), ration rich in concentrates (----).

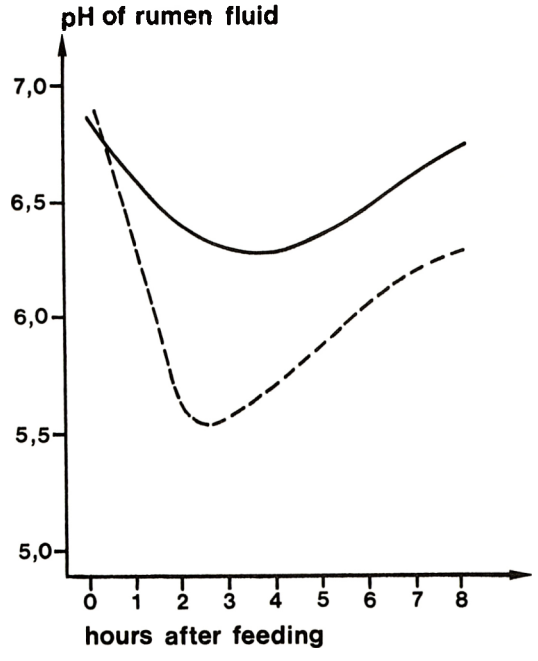
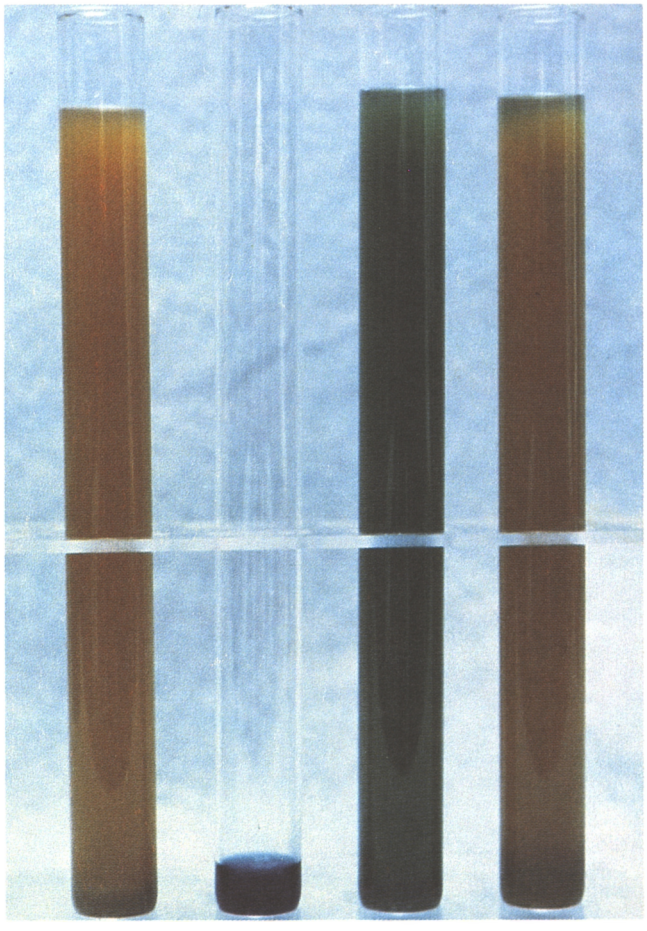


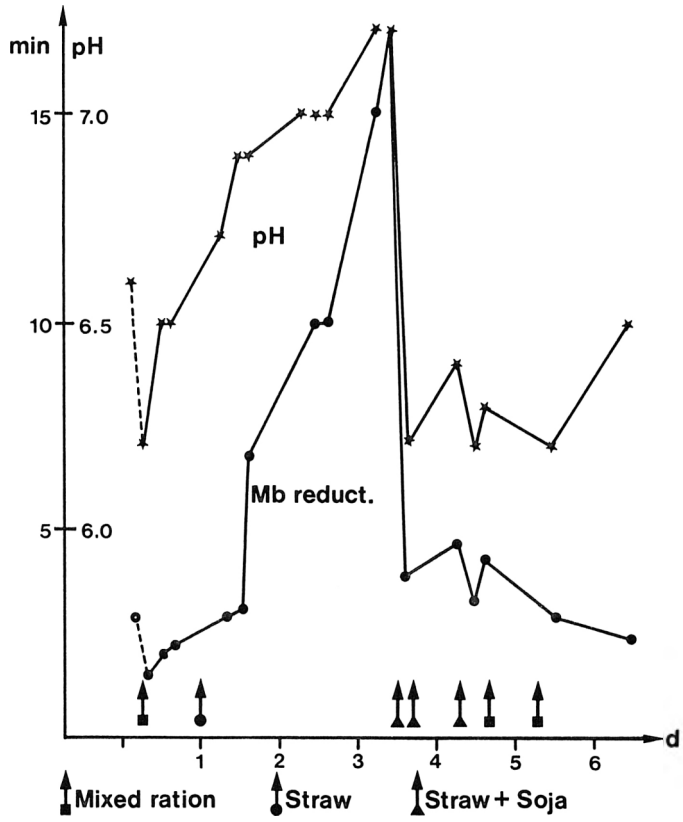
FIGURE 3. Methylene blue reduction: control tube, dye, tube immediately after addition of dye to rumen fluid, completed reaction.



to be interpreted carefully with due consideration for the degree of contamination with saliva during collection, as discussed below, and for the time since the last feeding (Figure 2). The pH generally rises above 7.0 within 12 hours after a meal rich in (fibrous) forages and within 24 hours after consumption of a mixed ration rich in concentrates. The pH is pathologically elevated above 7.0 by inanition and continuing saliva inflow, urea toxicity (rumen alkalosis), and putrefaction (due, for example, to consumption of spoiled feeds or feeds heavily contaminated with manure). The pH may be below 5.0 in acute rumen acidosis or below 5.5 in latent acidosis and when large quantities of abomasal fluid refluxed into the rumen exhaust the buffer capacity (Dirksen 1983).

A useful indicator of rumen fluid activity is the *redox potential,* as measured by the *methylene blue reduction* time. The redox potential is maintained by microbial fermentation, and to a lesser extent by enzyme systems of plant origin; the rumen is normally an anaerobic environment. The test involves mixing 1 ml of 0.03% methylene blue solution with 20 ml of fresh rumen fluid (at body or normal room temperature) in a glass test tube (Figure 3). If the sample

FIGURE 4. Effect of changing from a mixed ration to straw on methylene blue reduction time and rumen fluid pH three hours after feeding. Inactivation of the ruminal flora due to an energy poor diet is reversed by addition of soybean meal to the diet (Mahler 1970).



contains many greenish food particles in suspension, it can first be strained through gauze or cheese cloth to simplify detection of the blue color. The time for decoloration (to match a control sample, Figure 3) is inversely proportional to microbial activity. Highly active samples from cows fed a mixed hay and grain diet have a reduction time of 3 minutes or less. When cows are fed a diet consisting of mostly hay with some grain, 3 to 6 minutes are required. Relatively indigestible (straw) diets (Figure 4), inanition of several days duration, and rumen acidosis result in considerable delay in the reaction (Dirksen 1969). If a blue ring develops at the top of an otherwise decolorized sample, the reaction is judged to be finished; highly active samples generally have a narrow ring, inactive samples a broader blue ring.

When fresh rumen fluid is left undisturbed in a glass cylinder or test tube, the finer feed particles and large infusoria tend to settle, while larger fibrous constituents float to the surface (Figure 1). If the tube is held at body temperature, gas production by an active flora will eventually buoy to the surface some particles that originally sank. Watery inactive samples (starvation, inappetence, feed of low nutritional value, rumen acidosis) settle rapidly and little flotation occurs. When an animals consumes a rich, pelleted diet or when foamy bloat is present, the particles remain in suspension for a long time. If the holes in the collection instrument are relatively small, few large particles will be aspirated and thus the quantity of material that initially floats will be reduced. Straining the sample through gauze will also reduce flotation. Whether an attempt is made to judge the time until *sedimentation and flotation* are complete in a strained sample (Nichols and Penn 1958) or the percent of the height of the tube occupied by the sedimented material after 10 minutes (Elizondo-Vazquez 1975), the resulting value by itself usually does not allow differentiation between various forestomach diseases of cattle. It is, on the other hand, very useful to observe the sediment in the collection bottle or the methylene blue control tube for a layer of whitish-grey infusoria (Figure 1). When many infusoria can be seen with the naked eye, the sample can be assumed to be active. Absence of these organisms is not proof of inactivity, as only the largest species can be thus detected.

Both total *titratable acidity* and *chloride concentration* can be determined easily using kits designed for water testing (Weirather and Dirksen 1986). Total acidity generally increases as pH declines, and thus the information obtained is more confirmatory than diagnostic. Chloride concentration, on the other hand, is very important for the diagnosis of abomasalruminal reflux and will be discussed later in this paper.

Glucose fermentation is rarely determined except for research purposes, as there is a strong negative correlation between the quantity of gas produced and the simpler to determine methylene blue reduction time (Figure 5, Wenzel 1977). Microscopic examination of a drop of fresh, warm rumen fluid is very useful for judging the number and viability of *protozoa*. The presence of many active cilliates of various sizes is an indication of an active rumen; numbers are greatly reduced with inanition or diets lacking energy and protein. All protozoa die when the rumen pH drops to 5.0, and thus their absence in milky rumen fluid in an acute illness

FIGURE 5. Correlation between glucose fermentation (gas production) and methylene blue reduction time (Wenzel 1977).

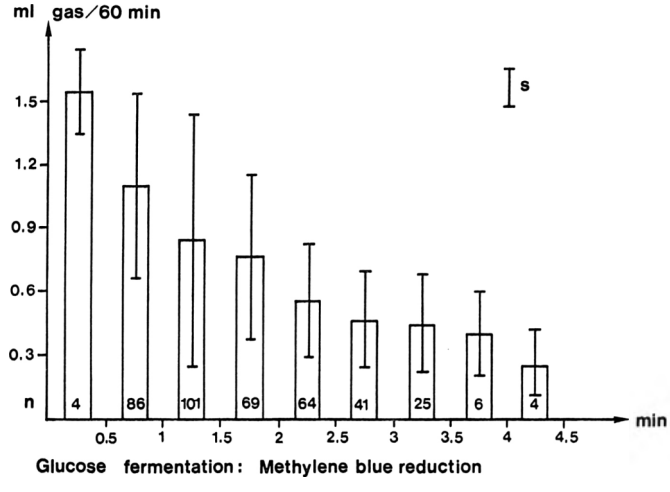


FIGURE 6a. Predominantly Gram-negative flora in normal rumen fluid.

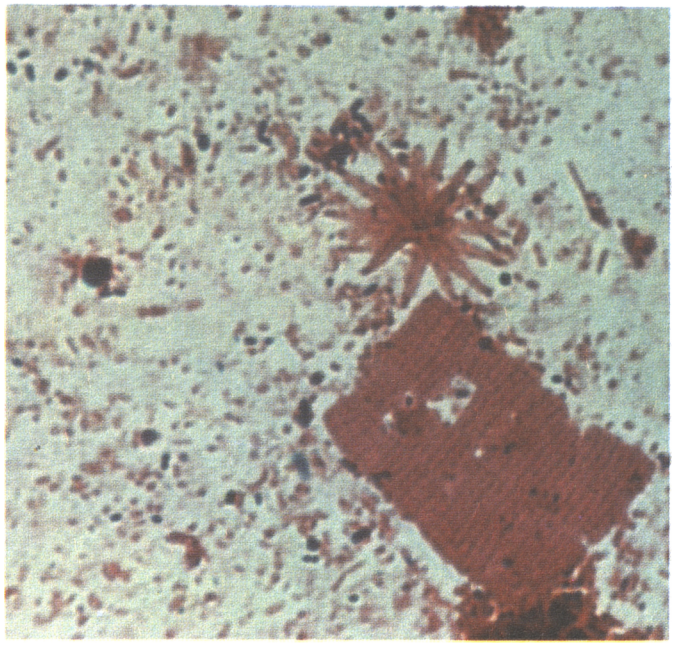
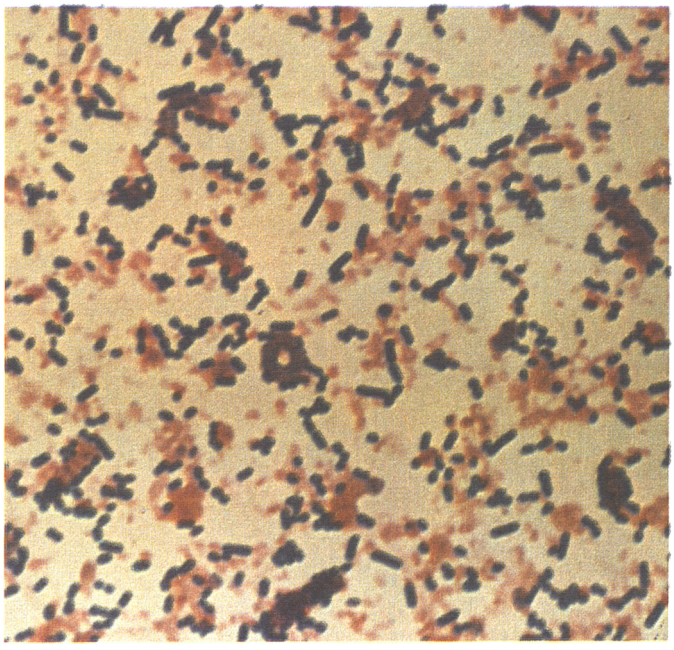


FIGURE 6b. Overgrowth of Gram-positive organisms with acute lactic acidosis.



is suggestive of rumen acidosis, even if the pH of the sample is in the normal range. An air-dried Gram-stained smear of strained rumen fluid examined under oil-immersion is also useful for confirming acidosis. The normal rumen *flora* consists of predominantly Gram-negative bacteria (Figure 6a), but overgrowth of Gram-positive organisms (first cocci, then lactobacilli) occurs with acute lactic acidosis (Figure 6b, see Dirksen 1977). When the sample is acid due to reflux of abomasal fluid, Gram-negative organisms predominate. The illustrations provided by Church (1976) are very helpful for identifying bacteria and protozoa commonly found in rumen fluid. Information concerning the other laboratory tests can be found in the writings of Dirksen (1977) as well as those of numerous other authors.

TABLE 4. Factors which influence the acquisition (quantity per unit time) and composition (influx of saliva) of rumen fluid.

Construction of the probe.
Composition of the ration.
Sampling time relative to last feed intake.
Fullness of the forestomachs.
Reaction of the animal.
Experience of the person obtaining the sample.

TABLE 5. Average values of 10 samples of mixed saliva from adult cattle (Wagner 1984).

	pH	Na+	K+	Cl−
		mmol/liter		
x	8.4	129	7.4	24.2
s	0.07	18.7	2.1	2.8
x min	8.25	79	2.6	19.0
x max	8.54	146	10.5	29.0

TABLE 6. Increase in the pH value of physiologic rumen fluid obtained per fistula, as a result of the addition of a 5% increment of saliva. (Wagner 1984).

Initial pH	5.5-6.0	6.1-6.5	6.6-7.0	7.1-7.5
⌀ x pH	0.17	0.12	0.10	0.07
x min	0.12	0.09	0.03	0.04
x max	0.25	0.15	0.14	0.13
n	20	22	60	40

TABLE 7. Concentration of Na+, K+, and Cl− (x mmol/l) after addition of saliva to samples of physiologic rumen fluid obtained per fistula (n=19). (Wagner 1984).

Saliva	0%	10%	20%	30%
Na+	93	98	103	108
K+	37	34	31	28
Cl−	25	25	26	25
Cl− max	35	34	33	31

Factors affecting the collection of rumen fluid

In order to obtain an adequate quantity of usable (e.g., not too heavily contaminated with saliva) rumen fluid, it is important to bear in mind a number of factors (Table 4).

Instruments: One should strive to aspirate fluid from the ventral sac of the rumen. Therefore, the probe for collection of rumen fluid from adult cattle must be at least 2.30 m long. The suction tube should have an internal diameter of at least 8 mm, as otherwise it frequently becomes plugged. Commercially-available instruments include the original (Figure 7) and the modified Schambye-Sörensen probe[c] and the guidable probe[d] (Dirksen 1977, Figure 8). The modifica-

[c] *Obtainable from Walter Eickemeyer, Veterinär-Instrumente, Eltastrasse 8, D-7200 Tuttlingen, West Germany.*

[d] *Obtainable from Firma Eisenhut, Sandweg 52, CH-4123 Allschwil, Switzerland.*

FIGURE 7. Original Schambye-Sorensen instrument.

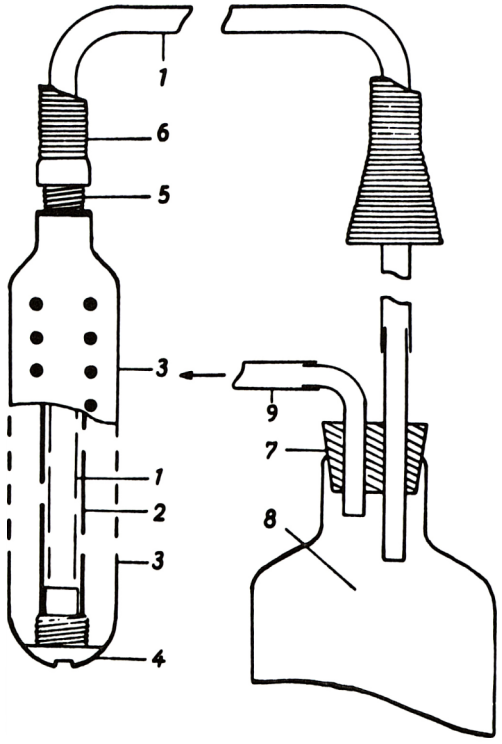


FIGURE 8. Guidable probe developed by Dirksen.

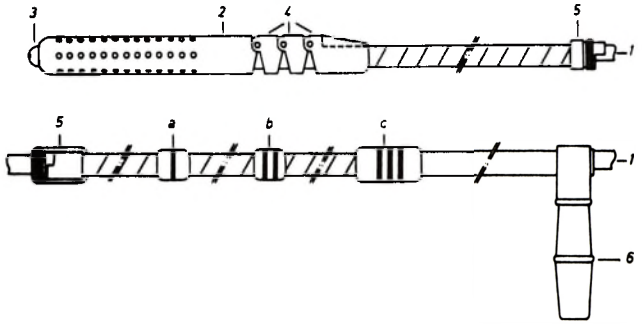


FIGURE 9. Variability in instrument flexibility, which is important relative to obtaining fluid from the ventral sac of the rumen. The less flexible instrument (above) is not suitable.

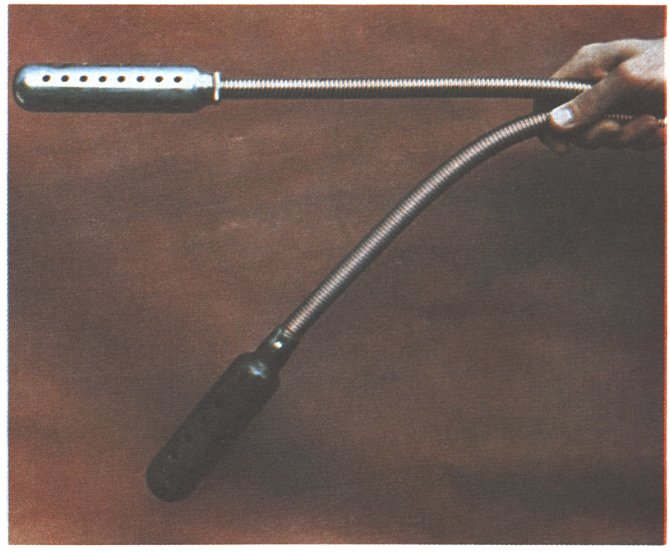
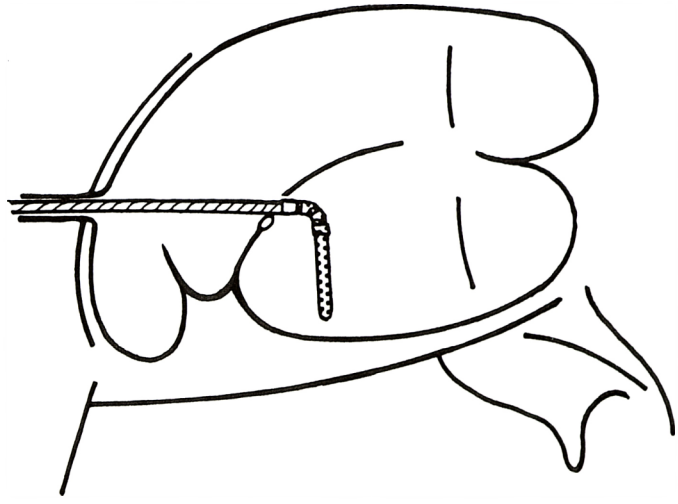


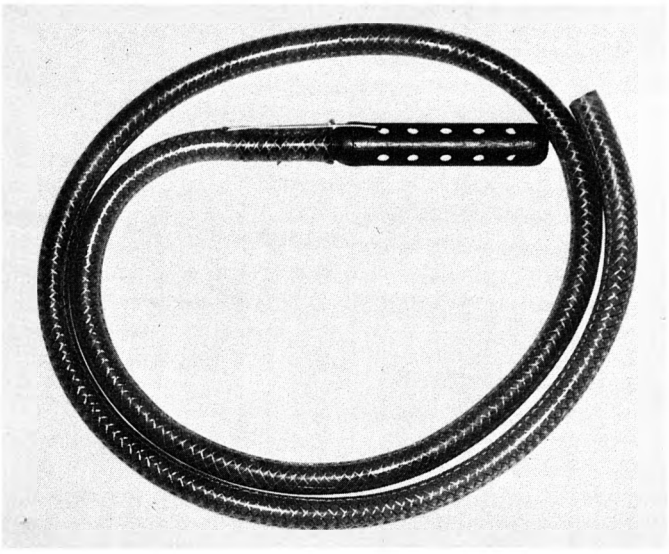
FIGURE 10. Proper placement of the suction head for rumen fluid collection.



tions that have improved the function of the original Schamby-Sörensen instrument include lengthening it, increasing the internal diameter of the suction tube, and eliminating a second filter tube within the suction head. The end of the instrument must be adequately flexible (Figure 9) and the suction head heavy enough that the ventral sac is entered. The guidable instrument is inserted with its handle pointing upwards until the anterior pillar is crossed, at which point the entire probe is rotated 180 degrees, thus permitting the suction head to dip into the fluid in the ventral sac (Figure 10).

A variety of other instruments have been used for collecting rumen fluid. One of the earliest devices proposed was a simple stomach tube passed through the mouth (Pounden 1954) or nose. Later modifications included aspiration with a large syringe to start the flow of fluid (Schulz and Hiepe 1958), the provision of many holes in the distal end of the tube (Holtenius, Björck and Hoflund 1959), and the temporary placement of a finger cot over the end of the tube to prevent entry of saliva during passage through the esophagus (Leek, 1983). Other authors describe perforated metal suction heads (Hull 1978, Keindorf and Link 1971, Steger, Voigt and Piatkowski 1968). The suction head of the instrument mentioned in Table 8 had an outer diameter of 2.4 cm and an internal diameter of 2.0 cm. The suction tube was proteced by a hard plastic outer tube and had multiple holes in the portion extending into the suction head. A final option for obtaining a small quantity of fluid is

FIGURE 11. Home-made equipment for obtaining rumen fluid from young stock.



simple needle aspiration of the caudoventral rumen sac. A small risk of localized peritonitis accompanies this technique (Hollberg 1984).

Instruments for obtaining rumen fluid samples from young calves and weanlings can be easily constructed (Figure 11). A perforated metal suction head with a diameter of 1.8 to 2.0 cm and a length of 9 to 14 cm is provided with 4-6 rows of holes having a diameter of 6 mm. The proximal 3 cm of the head is narrower (approximate diameter 12 mm) so that a plastic stomach tube can be tightly affixed. The wall of the suction head should not be too thin, or else the weight will be insufficient to carry it to the floor of the rumen. The device is passed through a wooden mouth gag.

The glass collection bottle is fitted with a rubber stopper through which two metal connectors pass. The suction tube from the probe or the stomach tube is attached to one metal tube. Suction is applied to the other by connecting it to the vacuum line in the dairy barn or a hand-operated pump.

Ration composition, time since last feed intake, and fullness of the forestomachs: When a ground (poorly structured) feed is provided, the normal separation between the ventrally located fluid accumulation and the overlying fibrous layer does not occur. Instead the rumen fluid is thoroughly blended with solid particles so that the probe easily becomes clogged. The proportion of fluid in the rumen contents is lowest immediately after feed consumption and greatest in the morning before the first feeding of the day. Early morning is thus the best time for obtaining large quantities of fluid for therapeutic purposes. The pH value of the rumen fluid decreases with increasing acid production via digestive processes and reaches its lowest point 3 to 5 hours after feed intake. Fiber-rich rations result in a higher pH plateau than those rich in starch or sugar.

Reaction of the animal and experience of the operator: The stronger the resistance shown by the animal to the passing of the probe and the less experience the person collecting the sample has, the longer the time that elapses between first introduction of the instrument and aspiration of rumen fluid. This results in greater contamination with saliva. The presence of a tube in the mouth and esophagus and pressure applied by its weight across the ruminoreticular fold stimulate a tremendous outpouring of saliva.

Effects of the parameters to be analysed of adding saliva to rumen fluid (in vitro experiments)

Depending on the previously mentioned factors, a variable quantity of saliva enters the probe during the collection procedure. The measured values for the parameters investigated deviate more or less from the real values of the rumen contents according to the quantity of inflowing saliva.

The pH value and electrolyte content of ten samples of mixed saliva obtained from the esophagus of adult cattle via permanent rumen fistula are presented in Table 5. Samples of rumen fluid obtained per fistula were combined in vitro with saliva. The proportion of saliva was increased in increments of 5% so that it contributed from 5 to 50% of the mixture (Wagner 1984). As is demonstrated in Table 6, the resulting pH value increased more markedly for samples with a lower initial value. This is related to a lower bicarbonate concentration (and therefore buffer capacity) in rumen fluid at lower than at higher pH levels (Kaufmann and Hagemeister 1969). The phenomenon is also partially explained by the fact that pH changes more slowly in the vicinity of the pK of the weak acid performing the buffering action (6.3 for carbonic acid, 4.75 to 4.81 for acetic, propionic, and butyric acids; Brugere 1984).

It can be concluded from Table 7 that the sodium concentration in rumen fluid increased linearly, and specifically by an aveage of 2.5 mmol/l, while the potassium concentration decreased by approximately 1.5 mmol/l each time the proportion of saliva in the mixture was increased by a further 5%. Because the chloride concentration in rumen fluid under physiologic conditions is approximately the same as in saliva, it was essentially unaffected by the addition of saliva.

The methylene blue reduction was slightly enhanced by the admixture of 5 to 10% saliva. It was delayed by proportionately greater volumes of saliva (12 sec longer at 15% saliva, 6 min 17 sec longer at 50% saliva) (Wagner 1984). There was a stepwise decrease in gas formation during glucose digestion with increasing proportions of saliva. Nitrite reduction also proceeded progressively slower. Further details can be found in the work of Wagner (1984).

Estimation of saliva contamination of rumen fluid samples collected via probe

The four different instruments listed in Table 8 were used to collect rumen fluid from eight healthy fistulated cows receiving various combinations of hay, silages and concentrates. A sample of rumen fluid was aspirated from the

TABLE 8. Instruments used for collecting rumen fluid in the present investigation.

Type	Length	Suction tube internal diameter
I Schambye-Sorensen, original	1.65 m	0.5 cm
II Schambye-Sorensen, modified	2.30 m	0.8 cm
III Dirksen,	2.30 m	0.8 cm
IV Plastic tube with metal head, self-produced	2.50 m	0.8 cm

TABLE 9. Differences (x) between the parameters measured in rumen fluid samples (n) obtained per probe and per fistula in investigations involving 8 fistulated cows (Wagner 1984).

Instrument / Parameter	I (n=20)	II (n=34)	III (n=34)	IV (n=20)
pH x diff.	+ 0.40	+ 0.25	+ 0.25	+ 0.28
Na x diff.	+ 10.0	+ 7.3	+ 6.5	+ 5.6
K+ x diff.	— 6.1	— 4.1	— 3.3	— 3.4
Cl— x diff.	— 0.9	+ 0.1	+ 0.2	+ 0.5

(Na+, K+, Cl— in mmol/l)

TABLE 10. Instrument-dependent contamination with saliva*, as calculated from the differences in pH, Na+ concentration and K+ concentration between "fistula" and "probe" samples. (Wagner 1984).

Instrument	I (n=20)	II (n=21)	III (n=21)	IV (n=20)
Average percent saliva	19.6	12.1 (14)#	9.9 (12)#	12.5
s	8.6	6.2	7.0	5.2
Minimum-maximum	6-35	3-29	2-30	2-20
Average collection time (sec/1)	67	48	36	45

* Experiments using 3 fistulated cows
# (Combined average for 34 samples from 8 fistulated cows)

TABLE 11. Collection time (sec) and average saliva contamination (%) associated with obtaining 1 liter of rumen fluid from 8 fistulated cows using Instruments II and III (Wagner 1984).

Collection time	Instrument II % saliva	(n)	Instrument III % saliva	(n)
< 60 sec	8.9	(22)	8.6	(13)
60-89 sec	13.9	(10)	13.5	(7)
90-139 sec	17.7	(6)	20.2	(2)
140-295 sec	23.3	(5)	25.6	(3)

ventral rumen sac via the fistula before each introduction of the rumen sound. Afterwards, the values of pH, K+, Na+, and Cl- ascertained in this sample were compared with those measured in the probe samples. The average differences are reported in Table 9.

Based on the in vitro changes in pH value as well as Na+ and K+ concentrations noted with each 5% addition of saliva, the degree of contamination with saliva was estimated from the differences between the test results from fistula samples and probe samples. The calculation was performed for each of the three parameters, and then the arithmetic mean of the three values was determined. The average value and range for percent saliva contamination with each instrument are given in Table 10. Although the average admixture of saliva determined for instruments II, III and IV varied from 12 to 14%, the estimated value for probe I (the original Schambye-Sörensen device) was 19.6%. Also noteworthy was the spread in the data, in as much as certain individual samples contained 30% or more saliva.

The dependence of saliva contamination on the duration of the collection is presented in Table II.

FIGURE 12. Effect of 12% saliva contamination on chloride concentration in rumen fluid: actual rumen fluid □, calculated value for probe sample ▨.

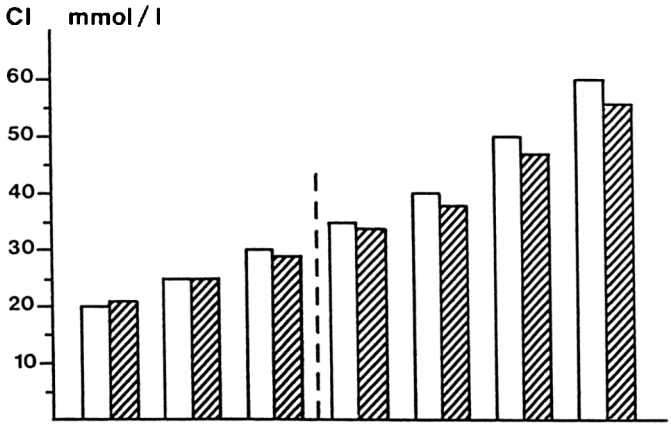
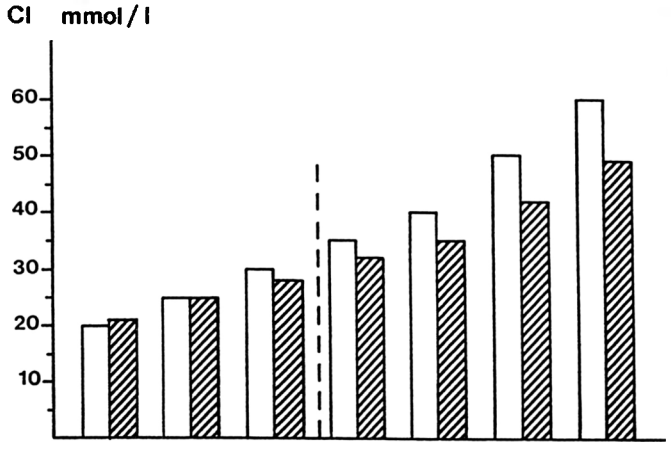


FIGURE 13. Effect of 30% saliva contamination on chloride concentration in rumen fluid: actual rumen fluid □, calculated value for probe sample ▨.



Diagnosis of abomasalruminal reflux

Given these results it should next be determined if contamination with 12 to 30% saliva would interfere with recognition of abomasalruminal reflux. In doing so it is accepted that the

upper physiologic limit for chloride in rumen fluid of healthy adult cattle is on the average 30 mmol/l (see Table 7). This of course assumes that the animal has not recently consumed large quantities of salt from a lick. Conditions that may be accompanied by reflux of abomasal fluid into the rumen include LDA, RDA, abomasitis, abomasal lymphosarcoma, vagus lesions (pyloric stenosis), incarceration of the abomasum, and peritonitis.

Figures 12 and 13 demonstrate the calculated influence of adding 12 and 30% saliva to rumen fluid containing various concentrations of chloride. As can be seen in Figure 10, the addition of 12% saliva causes a borderline chloride concentration of 30 mmol/l to decrease to 29 mmol/l, while 35 decreases to 34 mmol/l and 40 to 38 mmol/l. The effects of 20% saliva are similar. Incorporation of 30% saliva (Figure 11) would result in the following reductions from the initial chloride concentrations: 30 mmol/l decreases to 28 mmol/l, 35 to 32 mmol/l, and 40 to 35 mmol/l.

It is thus established that the diagnosis of abomasalruminal reflux by measurement of the chloride concentration in rumen fluid samples obtained through a probe is not influenced by contamination with saliva, because initial values that are clearly outside the normal range remain abnormal inspite of the reduction in chloride concentration caused by saliva. When the initial value is on the border between normal and abnormal, it should be kept in mind that higher proportions of saliva cause a proportionately greater decrease in the chloride concentration. Such values should be interpreted cautiously and the determination repeated after 12 to 24 hours.

Particulars concerning the usefulness of the instruments

It was important to determine that the collection of rumen fluid from adult cattle using probes of various designs or even very simply constructed instruments could be accomplished without special difficulties. However, differences could be demonstrated in the 'output rate' (that is, the quantity of rumen fluid aspirated per unit time) as well as in regard to saliva contamination. Thus collection using the original Schambye-Sörensen instrument resulted in a reduced output rate and an increased admixture of saliva as compared with the other three instruments. This is at least in part related to the relative shortness of the Schambye-Sörensen probe (1.65 m) in comparison with the other instruments (2.30-2.50 m) such that it only reached the ruminal atrium, and to the smaller diameter of the suction tube (0.5 as opposed to 0.8 cm). In addition, the experience of the operator, the temperament of the animal, and the type of ration play a more or less important role. Thus, for example, the feeding of high moisture ear corn rather frequently resulted in plugging of the suction head and thus a longer pumping time (and increased saliva admixture).

From the experimental results reported above it is possible to deduce the rule of thumb that aspiration of one liter of rumen fluid over 90 seconds or less using an instrument of type II-IV will result in a sample containing approximately 12 to 14% saliva. That the contamination with saliva would be decreased by discarding the first portion (ca. 200 ml) of aspirated rumen fluid was not verified in the present study but has been implied by other authors (Steger, Voigt and Piatkowski 1968). It appears more realistic to admit to an approximate admixture of 12% saliva.

When rumen fluid is harvested for therapeutic purposes, it is desirable to obtain it from animals on the same farm as the recipient, so that the microflora will be adapted to the diet being fed. This is not always possible. According to present knowledge, rumen fluid maintains adequate activity for up to 9 hours at room or refrigerator temperature (Dirksen and Wolf 1963, Mahler 1970). Neither the presence of saliva nor the changes that occur in microbial populations and fermentation products during transport will seriously decrease the therapeutic value.

It should be mentioned that the guidable probe is suited not only for collection of rumen fluid, but also for treatment of tympany accompanied by a free dorsal gas cap. For this purpose the suction tube is removed and replaced by a wire. Once the head of the instrument has been passed into the rumen, it can be directed upwards into the gas cap. Similarly, a tube with spray head is installed for treatment of foamy bloat. This permits dispersal of the appropriate medication throughout the rumen contents so that breakup of the foam occurs rapidly.

Conclusions

When evaluating a sample of rumen fluid that has been aspirated through a probe for diagnostic purposes, one should bear in mind that the nature of the sample can be influenced by admixture of saliva. Thus the pH of the sample is generally raised by contamination with saliva. The extent of the pH change depends on the one hand on the proportion of saliva, and on the other hand on the initial pH of the rumen fluid. The increase is most marked when the pH of the rumen fluid is low, and it becomes progressively smaller as the pH approaches that of saliva (8.4-8.6). Inasmuch as the contamination with saliva can be estimated to be approximately 12%-14% (as postulated above), a pH increase can be expected for samples in the acid range; the increase averages 0.4 to 0.2 units for samples initially below versus above a pH of 6.0.

With regard to the diagnosis of lactic acidosis, it should be noted that while the pH decreases to 5.0 or below at the time of maximal lactic acid production, the pH can rise to be within the physiologic range within the next 12 to 24 hours even though the rumen 'milieu' remains severely deranged. This occurs because of resorption and outflow of acid as well as influx of saliva into the rumen. Therefore, the diagnosis must not be based on the pH measurement alone. As long as the rumen fluid sample has the milky-grey color typical of lactic acidosis[e] (though its pH is in the normal range), the

[e] *So far, a similar milky color has only been observed after intraruminal application of relatively large quantities of caprylic (C_8), capric (C_{10}), or lauric (C_{12}) acid.*

suspicion of acidosis can usually be confirmed by means of a Gram-stained smear of rumen fluid. In addition, the feeding history (ingestion of large quantities of easily digestible carbohydrates) and the characteristics of the manure (yellow-brown, acid-smelling, loose feces with a pH < 8.0) as well as the pH of the urine (< 7.0) may give further indication for acidosis. On the other hand, when the absolute or relative pH value (in relationship to the time of last feed intake) is too low but the sample is a dark brown color rather than milky grey, then reflux of hydrochloric acid can be suspected. This can be confirmed by measurement of the chloride concentration.

The results of the various microbial activity tests mentioned—methylene blue reduction, glucose fermentation, and nitrite reduction—are essentially unaffected by addition of up to 15% saliva to rumen fluid. The same holds true for chloride concentration.

A careful evaluation of rumen fluid will help the practitioner to diagnose several important indigestions of cattle, including acute lactic acidosis, chronic-latent acidosis, abomasalruminal reflux, putrefaction, and inactivity of the microflora and fauna. Color, odor, consistency, pH, bacterial and protozoaal populations, microbial activity, and (especially important) chloride concentration can all be determined in a few minutes without sophisticated equipment. The added information gathered from these tests will often identify a particular problem. The findings of a normal, active rumen fluid is equally important, as this will help to rule out diseases involving the forestomachs. In summary, the routine clinical examination of cattle should include evaluation of rumen fluid.

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