Problem. Ruminants fed exclusively on succulent soft feed often develop a dangerous inflation of the rumen, resulting from accumulation of gas, a condition known as bloat. According to Cole, Mead, and Kleiber (1), who experimented on cows with rumen fistulae and made observations in the field, "Bloat is caused not by excessive gas formation but by interference with belching. It results most frequently from absence of the stimuli necessary for belching." An important condition for normal belching seems to be the presence of prickly material in the rumen. Dougherty (2) noticed that hydrogen sulfide or carbon monoxide injected into the tureen "caused a distinct paralysis of the organ when sufficient concentrations were reached." He found comparatively large amounts of hydrogen sulfide in gas and ingesta taken from a heifer that had died of bloat. Olson (3) reported later, "The amount of hydrogen sulfide in bloated animals may be ten to twenty times that of animals on dry feed" and "Alfalfa under certain conditions is very high in hydrogen sulfide." According to him, these facts suggest that "this gas plays a significant role in the cause and death from bloat in the bovine."

The experiments reported in this paper were carried out in order to investigate this possible relation between bloat and the chemical composition of rumen gases, especially the hydrogen sulfide content.

Experimental

Method. The storing of moist gas samples for determining small concentrations of hydrogen sulfide is complicated by the solubility of this gas in water or aqueous solutions and by its reaction with mercury. To avoid these difficulties, we measured the amount of gas (as taken directly from the rumen) that contains a given amount of hydrogen sulfide instead of using the ordinary method of measuring the amount of hydrogen sulfide in a given amount of gas. We thus measured the amount of gas which we had to pump from the rumen through 200 cc. of 0.001 N iodine solution for the reduction of the iodine, as indicated by the disappearance of the blue color that had been produced in the iodine solution by added starch. With relatively high concentrations of sulfide and iodine, errors occur because part of the sulfide is oxidized to sulfate instead of sulfur. Since, however, the hydroxide sulfide concentration in our absorbing liquid is very low and since also the iodine solution used was only 0.001 normal, this direct iodometric titration of hydrogen sulfide should produce correct results (4).

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The 0.1 N stock solution of iodine, prepared according to Treadwell (5), contained 12.7 g. iodine and 24.0 g. potassium iodide per liter. It was kept in the dark in a brown bottle. Just before the measurements in the field, 2 ml. of the stock solution was diluted to 200 ml. and 2 ml. of a solution containing 5 g. of starch per liter was added. The iodine solution was titrated with 0.1 N sodium thiosulfate solution.

Figure 1 shows the apparatus for pumping rumen gas through the iodine solution. Trocar $T$, with a side outlet, is inserted into the rumen of the cow.\(^1\) Stopcock $S_1$ is turned first so that the rumen gas bypasses the absorber, entering directly the 150-ml. measuring bulb $M$ while water flows from this bulb to the leveling bulb $L$. When the gas reaches the mark $m$ of the measuring bulb, stopcock $S_2$ is turned so that raising the leveling bulb expels the gas to outside. After 3 bulbs full of gas have thus been driven out, stopcock $S_1$ is turned so that the rumen gas, aspirated by lowering the

\(^1\) We obtained a sample of rumen gas from a bloated cow by a stomach tube inserted through the nose and then connected to our apparatus. For this operation we are indebted to Dr. John Britton, Division of Veterinary Science. The hydrogen sulfide content in this gas sample was 0.04 per cent. This value was not used for the calculation of our means.
leveling bulb $L$, bubbles through the iodine solution in absorber $A$, passing a double-action bubbler as shown in figure 1. The apparatus, mounted on an ordinary laboratory stand with elongated rod, was easily carried to the pasture where the rumen puncture was performed.

The number of bulbs full of rumen gas necessary to bleach the iodine solution, multiplied by the volume of bulb $M$ and by the temperature-pressure factor, indicates the amount of rumen gas that reduces the 0.2 milliequivalent of iodine in the absorbing liquid. We assume that the reducing agent is hydrogen sulfide alone, and thus the reduction of 0.2 milliequivalent of iodine indicates 0.1 millimol or 2.2 ml. hydrogen sulfide.

The following example illustrates the procedure of calculation:

Cow 624. Time: October 16, 1942; 2:30 P.M. Condition: Not bloated. Test No. 3.

Number of strokes of pump for bleaching iodine solution: 13.

Volume of measuring bulb, 144 ml.; reduced to standard condition, 130 ml.

Volume of rumen gas bubbled through absorber: $0.130 \times 13 = 1.7$ liters.

According to calibration with Na$_2$S$_2$O$_3$, the iodine solution contained $0.85 \times 0.2 = 0.17$ equivalents of I.

Reduction of this amount by H$_2$S indicates $0.85 \times 2.2 = 1.9$ ml. H$_2$S.

Since 1.7 liters rumen gas contain 1.9 ml. H$_2$S, 0.1 liter = 100 ml. rumen gas contains

$\frac{1.9}{1.7} = 0.11$ ml. H$_2$S.

At the end of 3 such measurements of the hydrogen sulfide content, a sample of the sulfide-free gas was kept in bulb $M$ and later analyzed for carbon dioxide, methane and oxygen in a standard Burrell gas-analysis apparatus.

In two such analyses rather low methane and correspondingly high nitrogen values were obtained. We suspect that in these two cases the platinum wire for combustion of methane was kept at too low a temperature and that this condition resulted in incomplete combustion. This explanation is strengthened by an abnormal combustion quotient: $\frac{\text{CO}_2 \text{formed}}{\text{O}_2 \text{used up}}$ of 0.13. For methane combustion this quotient should be 0.50. The average quotient of our normal analyses was actually $0.515 \pm 0.017$ ml. carbon dioxide per ml. oxygen.

The cows from which the gas samples were taken were pastured daily on alfalfa together with a group of 5 to 17 other cows. All were in lactation. They received concentrates at night. On 5 nights concentrates were the only supplement to the alfalfa pasture. Under those conditions at least 1 in 16 cows bloated on the alfalfa pasture next morning. On two days following the nights without roughage, gas samples were taken from bloated cows; on 3 days from cows that were not bloated. One night the cows were given fine alfalfa hay. The next day 1 of 15 cows bloated on the alfalfa pasture. That day's gas sample was, however, taken from a cow that was not bloated.
On two nights the cows had access to Sudan hay in addition to their normal ration of concentrates. There was no bloat on the alfalfa pasture on either of the following days, on each of which a sample of rumen gas was taken.

**Results.** Table 1 shows the results from these trials. The data are arranged approximately according to the intensity of the bloat, as observed when the gas samples were taken and as measured by the ratio of bloated cows to total cows on the pasture. The concentration of hydrogen sulfide in the rumen gas ranges from 0.08 to 0.16 per cent. It shows no correlation to the intensity of bloat. The mean of the 8 trials is $0.11 \pm 0.01$ per cent hydrogen sulfide.

This concentration is considerably above those observed by Dougherty (2), who reports “as much as 0.03 per cent hydrogen sulfide in rumen gas.” Dougherty found an exceptionally high value of 0.15 per cent in the rumen gas taken from a heifer that had died of bloat. This concentration is within the range of our results obtained on nonbloated cows.

**TABLE 1**

*Composition of rumen gas from cows on alfalfa pasture*

<table>
<thead>
<tr>
<th>Date</th>
<th>Roughage fed night before gas sample was taken</th>
<th>Condition of cows</th>
<th>Composition of rumen gas volume per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of cows on pasture</td>
<td>Number of cows bloated</td>
<td>Cows from which gas sample was taken</td>
</tr>
<tr>
<td>1942 Oct. 27</td>
<td>No</td>
<td>6</td>
<td>3 Bloated</td>
</tr>
<tr>
<td>26</td>
<td>“&quot;</td>
<td>6</td>
<td>2 &quot;&quot;</td>
</tr>
<tr>
<td>15</td>
<td>“&quot;</td>
<td>16</td>
<td>6 Not bloated</td>
</tr>
<tr>
<td>23</td>
<td>“&quot;</td>
<td>13</td>
<td>2 “&quot;</td>
</tr>
<tr>
<td>10</td>
<td>“&quot;</td>
<td>16</td>
<td>1 &quot;&quot;</td>
</tr>
<tr>
<td>14 Fine alfalfa hay</td>
<td>15</td>
<td>1 “&quot; “&quot;</td>
<td>0.16</td>
</tr>
<tr>
<td>8</td>
<td>Sudan hay</td>
<td>18</td>
<td>0 “&quot; “&quot;</td>
</tr>
<tr>
<td>16</td>
<td>“&quot; “&quot;</td>
<td>16</td>
<td>0 “&quot; “&quot;</td>
</tr>
</tbody>
</table>

All our cows were on alfalfa pasture. Possibly cows on other pasture or on dry feed have a smaller percentage of hydrogen sulfide in their rumen gas. Our results demonstrate, however, that the relatively high hydrogen sulfide concentration in rumen gas of cows on alfalfa pasture does not necessarily lead to bloat. Most likely, therefore, hydrogen sulfide concentration plays no very significant role as a condition producing bloat. Conceivably, on the other hand, this poisonous gas is involved in the fatal consequences of bloat. Any poison that destroys blood and tissue pigments involved in respiration would be particularly dangerous in a condition such as bloat, which interferes mechanically with normal respiration. Even the accumulation of carbon dioxide in the rumen is much more disastrous than the accumulation of an equal pressure of methane or oxygen (1).
Only three samples of rumen gas were completely analyzed. The concentrations of carbon dioxide and methane are within the range of results obtained earlier (1). The concentration of oxygen in the rumen gas was very low, a fact that also confirms previous observations.

SUMMARY

The hydrogen sulfide content of the rumen gas of bloated and nonbloated cows on alfalfa pasture was measured in 8 trials by an iodometric method especially adapted for this purpose.

The rumen gas contained, on the average of 26 titrations, 0.11 ± 0.01 per cent hydrogen sulfide by volume. There was no relation between hydrogen sulfide concentration and bloat.

The rumen gas contained on the average 67 per cent carbon dioxide, 26 per cent methane, and less than 1 per cent oxygen. The concentration of these gases was not related to bloat.

This result supports the theory that bloat is caused, not by abnormal gas formation, but by a lack of belching.

ACKNOWLEDGMENT

We are grateful to Mr. Th. Chernikoff for his valuable help in taking the gas samples and in their analysis.

REFERENCES

(4) KOLTHOFF. Volumetric Analysis II. Transl. by H. Fuhrmann, 398. 1929.