to an identically treated standard. It should be mentioned that the ketoses require a longer development-time than the aldoses and the disaccharides an even longer time. Experiments with a varied development-time has disclosed that a maximum reduction value is reached after 5 minutes in the case of the monoaldoses, after 10 minutes in the case of the ketoses, and after 10—12 minutes in the case of the disaccharides. However, even at the maximum value, the difference between aldoses and ketoses is retained. This fact is in agreement with the results of experiments using the method of Wallenfels *et al.*[3], where fructose and sorbose required treatment in steam for 1.5 minutes whereas a greater amount of aldoses could be developed in 10—20 seconds. The longer development-time required in the case of the ketoses can be explained on the basis of the chemical structure and mode of reaction. It is more difficult to explain why the disaccharides require such a long time for complete development. It could be expected that the mode of reaction of glucose and maltose is identical and that the reduction value per weight of the disaccharide is half that of the monosaccharide. This relation is obtained after 12 minutes development (Fig. 2b). With these extended development-times the background becomes correspondingly higher and the values therefore less reliable. — When the method of Wallenfels *et al.*[3] is applied, the spots of maltose and glucose are developed at equal rates.

A complicating factor is the experience that, in washing with 6 N NH_3, a part of the Ag is extracted. This is particularly evident in the case of tri- and oligosaccharides, where the washing produced a total decoloration of the spots. Since this extraction does not occur in 2 N NH_3 it seems most likely that the extraction is due not to the presence of Ag_2O but to the oxidation and dissolution of the finely dispersed Ag.

Conclusion: ^{110}Ag can be used for chromatographic determinations of reducing sugars. The isotope is useful for investigations of reaction conditions in chromatographic methods involving development with Ag. The fact that di- and monoaldoses give reduction values proportional to the number of reducing groups (Fig. 2b), indicate the possibility of estimating oligosaccharides even in cases where standards can be obtained only of the mono- and disaccharide of the series.

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Ruminant Bloat and Foaming Activity of Clover

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Many explanations have been proposed for ruminant bloat, in which thousands of cows die each year. As the bloating can be stopped, and the animal cured with anti-foaming agents as oil if given in good time, it is most likely that bloat is caused by the intense formation of foam in the rumen. Solid particles from the contents of the rumen gather into this foam, and the passage for gas escape becomes accordingly blocked. The great amounts of gas which are normally formed by fermentations in the rumen thus cause the bloat.

In order to find out if a great amount of substances with strong foaming activity are found in red clover which in Finland is the most dangerous cause of bloat during the latter part of summer, we made some determinations on the foam producing qualities of clover and timothy. The method used was the following.

Measurement of foam produced by shaking of crushed samples of plants in water. Parallel experiments with different amounts of water showed that a 5 % suspension of crushed fresh clover in water was the most satisfactory concentration for examination

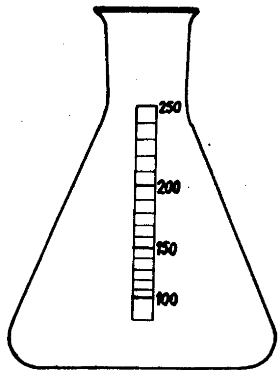

Fig. 1. Markings scratched on glass.

of foaming. The plant material was crushed as much as possible in the same manner in all the experiments. 100 ml water + 5 g crushed plants was put in a 250 ml flask, and the flasks were shaken in a flask shaker, always at the same speed (approx. 300 per min). The froth produced was quite stable, approx. 1/3 of the volume still remaining after standing overnight. Figures on the volume of the foam were fairly well reproducible for triplicate samples.

In all experiments a 5 % suspension was used. Graphs were plotted against time of shaking at constant speed. Volume of froth was read off directly from the graduations marked on the flasks (Fig. 1). All samples were done in triplicate.

Maximum amount of foam was usually produced during 1—3 hours of shaking. In some cases it was equivalent to the foam produced in parallel experiments by a 0.01 % solution of Baker saponin.

Fig. 2 shows the formation of foam in different experiments. All the investigated samples of red clover contained a great amount of foam-producing substances. As a general tendency we may, however, note that the samples taken in August have had a greater foaming activity than those taken in September. In the last samples (19/9) the activity has been only about half of that in the samples from August. It is to be observed that the pasture season in Finland generally ends in the middle of September. It is unfortunate that we have no determinations of red clover from the earlier part of summer when bloat is very scarce.

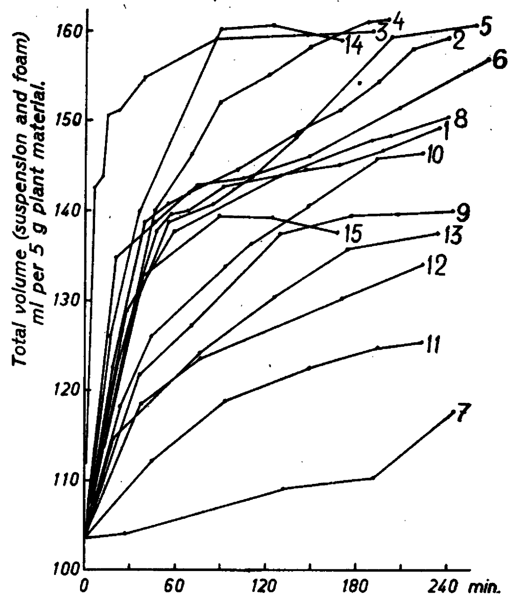

Fig. 2. Foaming of crushed clover, grass, and hay.

1. Red clover, 13.8.1952;
2. Red clover, 15.8.1952;
3. Baker saponin, 0.01 % solution;
4. Red clover, 21.8.1952;
5. Red clover (2nd year, 3rd crop), 27.8.1952;
6. Red clover (1st year, 3rd crop), 27.8.1952;
7. Grass, 3.9.1952;
8. Red clover (1st year, 3rd crop), 3.9.1952;
9. Red clover (1st year, 3rd crop), 11.9.1952;
10. Red clover (2nd year, 3rd crop), 11.9.1952;
11. Grass, 11.9.1952;
12. Red clover (2nd year, 3rd crop), 19.9.1952;
13. Red clover (1st year, 3rd crop), 19.9.1952;
14. Hay (clover), 12.12.1952;
15. Hay (timothy), 12.12.1952.

The few samples of fresh grasses (mostly timothy) studied (7, 11) showed much lower foaming activity than fresh clover. With hay of clover and timothy — when the high drymatter content (about 90 %) of hay (expts. 14 and 15) compared with fresh clover and timothy (drymatter content about 20 %) is taken into account —

much less foam was formed than with corresponding fresh samples. In addition the froth which had been formed with hay of both types was much less stable, and commenced subsiding immediately after removal from the shaker, the subsidence being almost complete after about 1 hour. These observations agree with the well known fact that grass without leguminous plants does not cause bloat, and that clover made into hay does not do it either. The untenableness of the froth formed with hay is an interesting phenomenon, and suggests that in addition to the decrease of the saponin content also other changes influencing the quality of the foam occur in the drying of clover.

The attempt to produce bloat experimentally with two cows by giving them about 25 g of commercial saponin failed. We did not therefore publish our abovementioned results, as more knowledge about the substances with foaming activity in clover was needed, as well as animal experiments. The commercial saponin was apparently as foam-producing as that found in clover (*cf.* curve 3).

The present publication of our results depends on that Lindahl *et al.*[1] have recently published a communication about their preliminary investigations on the role of alfalfa saponin in ruminant bloat. They have effected bloat in sheep, goat, and heifer by saponin isolated from this leguminous plant. The amounts of alfalfa saponin which they have used in animal experiments (15—55 g to sheep, and 75 g to heifer) are considerably greater than the amount of commercial saponin which we gave to our cows, so that our negative result may depend on this. It is, however, noteworthy that Lindahl *et al.* did not effect any perceptible bloat in sheep with commercial saponin (from the yucca plant).

Our observations about the great amounts of foam-producing substances (or substance) in fresh clover, and their scarcity in fresh grass (timothy), and in hay made of clover and grass give good support to the conception that bloat in ruminants is caused by an intense formation of foam in the rumen, and that this formation prevents the escape of gas from the rumen. The animal experiments of Lindahl *et al.* with alfalfa saponin have proved the correctness of this idea.

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On the Formation of Phlorizin in Normal- and Low-Nitrogen Apple Maidens

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In this laboratory, the influence of the nitrogen content of cells on their enzymatic activity with microorganisms has been previously investigated. It then appeared that some enzymes lose a large, or even the largest, part of their activity when the N-content of the cells falls by 20—40 %, while other enzymes retain their activity rather well. The mutual relation between the enzymes in a low-N cell thus differs entirely from that in normal-N cells. With *Aerogenes* bacteria for instance, Virtanen and Alonen [1] noted that low-N cells form fermentation products from sugar in quite different proportions than normal-N cells.

One of us [2], has recently given an account of investigations on the influence of nitrogen fertilizing on the nitrogen content, and various nitrogen fractions, of apple maidens. These investigations showed that the maiden can grow relatively well during one summer without N-nutrition, although the nitrogen content falls to about half that of the maiden grown with nitrate. In the roots, the drop in nitrogen is even greater (*cf.* Table 1). It is thus possible to investigate the influence of the lowering of the nitrogen content on the metabolic reactions and enzymatic activity with higher plants as well.

Table 1 shows analyses of different nitrogen fractions in maidens, stocks and roots of the apple trees. Plant 5 was given no nitrogen fertilizers and the leaves of Plant 2 were sprayed with urea solution eight times during the summer. However, this did not influence the N-content of the plant, so that both Plant 5 and Plant 2 represent low-N plants. Plants 12 and 18 were fertilized with nitrate, which they readily absorbed, as can be observed from the nitrogen analyses of the plants. These plants represent high-N plants. The growth of the plants appears on Table 2.

When apple maidens were extracted with 70 % alcohol and the alcohol evaporated, we observed a copious crystalline precipi-