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 above-mentioned 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-

Table 1. Composition of N-fractions in "Maiden", "Stock", and "Roots" of appletrees without and with nitrate (mg N/100 g. dry matter).

Plant N:o	Plant part	Total org. N	"Protein N"	Soluble N	Amino N	Amide N
5	Maiden	642	575	67	36.5	9.9
(No N)	Stock	328	296	32	18.6	4.8
	Roots	413	380	33	19.9	4.4
2	Maiden	536	470	66	31.5	8.8
(Urea)	Stock	313	282	31	18.3	5.8
	Roots	455	425	30	19.7	5.6
12	Maiden	1 000	806	194	91.6	55.1
(NO_3)	Stock	969	651	318	129.8	85.3
	Roots	1 608	984	624	225.5	164.1
18	Maiden	891	699	192	61.2	28.2
(NO_3)	Stock	971	691	280	105.8	70.7
	Roots	1 521	1 009	512	193.1	133.6

tate which had been formed in the extract of low-N stocks and roots. In corresponding extracts of high-N plants, a precipitate of this kind was not observed. The precipitate was separated and recrystallized several times, its melting point being 114—115° C (uncorrected). This substance did not contain any nitrogen and gave in analysis 53.02 % C and 5.90 % H. It contained an aromatic ring, phenolic hydroxyl groups, and a CO group, and from the molecular weight determinations, seemed to contain 2 benzene rings with 3 hydroxyl groups. The CO group apparently belonged to the carbon chain which linked the rings. At this stage we noticed that phlorizin is a known compound in the bark of the apple tree and the mixed melting point and other characteristics showed our substance to be identical with phlorizin. The paper chromatograms (*cf.* below) were also identical.

Quantitative determinations showed that 5.2 % of phlorizin on a dry weight basis, could be found in low-N stocks, and 1.2 % in high-N stocks. Lack of plant material prevented us from further quantitative determinations of phlorizin. According to approximate observations during the evaporation of ethanol, the lower value may be valid for stock and roots of all high-N plants, while the higher value is valid for the corresponding parts of all low-N plants.

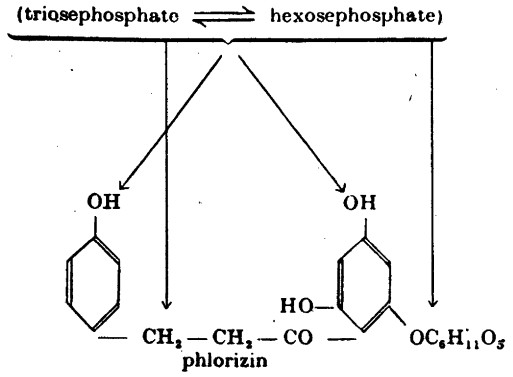
It is possible to separate and identify phlorizin using paper chromatography. With 70 % ethanol we found the R_F value to be 0.82, and with phenol-water 0.69. Chromatograms were developed by spraying with benzidine, prepared according to Horrocks [3]. Spots appeared upon warming at 100° C for 10—15 minutes.

As phlorizin is formed most freely in low-N plants it is probable that nitrogen deficiency brings about changes in the mutual relations between the enzymes in apple maidens, in consequence of which the phlorizin synthesis increases. Phlorizin is formed from the products of CO_2-assimilation, and it can be calculated that

Table 2. Initial weights of plants and production during experimental time.

	Plant				
	5 (No N)	2 (Urea)	7 (Urea)	12 Nitrate	18 Nitrate
Initial weight of rootstocks, g. dry m.	34.3	30.4	46.6	23.0	28.8
Increase in rootstocks, g. dry m.	4.8	7.9	5.3	8.5	5.3
Weigth of maidens, g. dry m.	6.7	7.7	6.9	11.1	10.0

3.5 hexose molecules are needed to form one phlorizin molecule, irrespective of whether the synthesis occurs from the triose, or earlier, stages, or from the hexose stage.



Accordingly, the increased phlorizin synthesis brings about a decrease of sugar and of soluble carbon compounds in general in the plant sap, since phlorizin is very difficult to dissolve in water. This is obviously of importance for the plant, since lack of nitrogen has reduced the normal synthesis of amino acids to a minimum, and the utilization of carbon compounds in amino acid synthesis has thus become smaller.

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Removal of an *Iso*propylidene Group in 1,2—5,6-Di-*iso*propylidene-D-glucofuranose with a Sulphonic Type of Resin

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α-1,2-*Iso*propylidene-D-glucofuranose is a very useful compound for the preparation of other derivatives of D-glucose. In the past, it has usually been prepared from 1,2—5,6-di-*iso*propylidene-D-glucofuranose by careful hydrolysis with dilute aqueous hydrochloric acid [1], aqueous acetic acid [2] or nitric acid and ethyl acetate [3]. The preferential removal of the 5,6-*iso*propylidene group is due to its greater susceptibility to acid as compared with the other.

In some synthetic work carried out in this laboratory it was found that the preferential removal of the 5,6-*iso*propylidene group in the di-*iso*propylidene derivative was effected in a few hours at room temperature on treatment with a sulphonic type of resin. This method which gives a good yield of the desired compound is also very convenient.

The use of an acidic resin for the hydrolysis of sugar derivatives has been reported previously, *e. g.* the hydrolysis of alkyl glycosides [4], N-glycosides [5] and now recently for the removal of an *iso*propylidene group in the synthesis of some methylated galacturonic acids [6].

Experimental. 1,2-5,6-di-*iso*propylidene-D-glucofuranose (8 g) was dissolved in water (320 ml) and Amberlite IR 120-(H) (16 g in the hydrogen form) were shaken at room temperature for 3.5 hours. The resin was filtered off, washed with water, the filtrate concentrated in vacuum to dryness and a white material was obtained (6.7 g). It was recrystallised from ethyl acetate. Small amounts of insoluble material, which was probably D-glucose and starting material, were filtered off. The recrystallised material (4.6 g) had m.p. 156—158° C and $[\alpha]_D^{20} = -11.3°$ (*c*, 1.25 in water). The literature reports m. p. 156—157° C and $[\alpha]_D^{20} = -11.8°$.

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