Bloat in Cattle

MUCH experimental work has been done on the causes of legume bloat in cattle and the prevention of its occurrence in the field (for reviews see Johns¹). Several substances have been isolated from the feed or the animals' saliva, and have been reported to be responsible for the foam in the rumen of a bloated cow; but it has not been shown that any of these substances occur in the foam found in the rumen, and no attempt has been made to isolate the surface-active compound responsible for the foam directly from the rumen contents of a cow grazing clover or lucerne.

In my experiments, serial samples of rumen fluid were taken from cows through a permanent fistula, commencing before the beginning of either eating freshly cut clover and lucerne in the stall or grazing a new area and continuing for 6 hr. The pH, ϵ H, total volatile fatty acid and ammonia nitrogen of the samples were determined. The remaining liquor was treated with 'Hyflo Supercel' to aggregate the suspended particles, and the straw-coloured filtrate was collected at the pump. The filtrate was found to foam considerably on shaking. The constituent responsible for the foam was not precipitable by mercuric chloride in the hot and was unaffected by a few minutes boiling with nitric acid or sodium hydroxide, but was decomposed by boiling for $\frac{1}{2}$ hr. with 3 per cent nitric acid. It did not pass through a dialysis membrane. The foaming constituent was precipitated from solution by the addition of ethanol to a concentration of 70 per cent at pH 4. After centrifuging off, the ethanol precipitate was redissolved in water to give a solution which foamed on shaking, indicating that the compound was not denatured by the alcohol precipitation. These results suggested that the constituent was carbohydrate in nature. The ethanol-precipitated material was hydrolysed for 4 hr. with 3 per cent nitric acid free from nitrogen peroxide containing a crystal of urea and the neutralized hydrolysate chromatographed on filter paper. The main constituents were the sugars normally found in the pectin and hemicellulose fractions of plants.

In each of the three experiments it was found, by using an accurate empirical laboratory method, that maximum foaminess occurs in the rumen 1–2 hr. after the cow began eating clover or lucerne, and fell away 4 hr. later to the pre-feeding level; the rumen contents of these cows were more foamy at all times than the contents from any cow eating grass or winter rations. This observation suggested that the foaming constituent appeared in solution in the rumen liquor after the plant had been physically broken down, and that it later disappeared, probably by the degrading action of the micro-organisms.

The whole picture of the fermentation, in the rumen of a cow eating clover or lucerne, is of a rapid reaction, as indicated by the high volatile fatty acid and ammonia nitrogen concentrations with copious gas production, the latter being entrapped in small bubbles in the rumen liquor owing to the presence of the pectins and/or hemicelluloses. It is important to note that under these feeding conditions, both the requirements for foaming to occur, namely, high gas production and the presence of a surface-active agent, are satisfied together and bloating may therefore occur. Each factor occurring on its own in this or any other situation may have no detrimental effect on the animal. The postulation that plant pectins and hemicelluloses are the substances respons-

ible for the formation of a foam in the rumen of bloating cows does not preclude absolutely that other factors may be involved.

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¹ Johns, A. T., *Vet. Rec. Annot.*, 2, 107 (1956); 4, 17 (1958).

PLANT PHYSIOLOGY

Effects of Auxins on Membrane Permeability and Pectic Substances in Bean Endocarp

It has been shown^{1,2} that auxins delay the onset of senescence in bean endocarp and various leaf tissues (*Rhoeo*, *Mesembryanthemum*) maintained *in vitro*. In the absence of auxin, liquid-logging of intercellular spaces is followed by cellular dissociation and rounding off of cells. Similar changes have been observed in the abscission zones of *Coleus*, *Cestrum* and *Fuchsia*. When de-seeded bean pod segments are maintained in Petri dishes on wet filter paper, liquid-logging is evident in 2½–3 days and an exudate appears on the upper tissue surfaces. Cellular dissociation is usually complete by the fifth day. These changes are essentially prevented by the addition of an auxin (3-indoleacetic acid or α -naphthaleneacetic acid) to the culture medium. There are no significant differences in increase in fresh weight or respiratory activity between water- and auxin-treated endocarp segments over a ten-day period. The cells of liquid-logged segments are still capable of plasmolysis and deplasmolysis. The results indicate that the liquid-logging and exudation are manifestations of changes in permeability. A second process involves changes in the composition of the cell-wall cementing substances which take place in the absence of auxin and give rise to cellular dissociation and rounding off of cells.

Previous studies^{3–6} have implicated pectin methyl-esterase as one of the enzymes directly affected by auxins. For pectin methyl-esterase, auxin appears to act through an adsorption-desorption mechanism which controls the activity of the enzyme in the tissue.

The results presented herein concern the nature of the permeability changes and the role of pectic enzymes in the processes causing cellular dissociation. Bean segments were prepared as before², and the exudate from water-treated segments collected for analysis. On addition of 3 vol. of ethanol, a precipitate was formed which was collected by centrifugation. The precipitate was dissolved in alkali, reprecipitated by acidification, and digested with a commercial pectinase preparation. The only substance detected in the digest was galacturonic acid. Analysis of ethanol-soluble substances in the exudate showed the presence of amino-acids, traces of organic acids and a large amount of a non-reducing sugar, which was identified as sucrose by paper chromatographic methods as well as identification of the hydrolytic products. Quantitative determination of the sucrose in the earliest-obtainable exudate gave a figure of 0.029 M. The osmotic pressure of the same exudate was found to be 0.050 M, thereby showing that sucrose is the major osmotic constituent. Ash