

the oxalate method to be in the region of 0.14 p.p.m.<sup>2</sup>. A symptom of the deficiency which does not appear to have been previously recorded is the production of bell-shaped leaves (Fig. 1). These would appear to result from the fusion of the lamina of basal leaves followed by abscission of the growing point. The level of molybdenum at which particular varieties of Brassicas become affected by whiptail is at present under investigation.

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<sup>1</sup> Walsh, T., Neenan, M., and O'Moore, L. B., *Nature*, **170**, 149 (1952).

<sup>2</sup> Walsh, T., Neenan, M., and O'Moore, L. B., *Dep. Agric., Dublin*, **47**, 3 (1952).

### A Remarkable Disintegrative Effect of Skatole upon Certain Rumen Ciliate Protozoa

Hogg and Elliott<sup>1</sup> noted that indole and skatole were both highly toxic towards the aerobic free-living ciliate *Tetrahymena pyriformis*. A concentration of 0.01 per cent of indole, for example, killed all organisms in a vigorous culture in two hours, but these workers made no mention of any characteristic change in appearance of the cells. We have now shown that these substances are highly toxic to the anaerobic ciliates of the sheep's rumen, particularly the holotrichs (*Isotricha* and *Dasytricha*) and also *Ophryoscolex*. These ciliates had been separated from strained rumen contents, washed and maintained alive and active for several hours in a phosphate buffer (without antibiotic) at pH 7.2 as described by Heald, Sugden and Oxford<sup>2</sup>. On a molecular basis skatole was much more toxic than indole to the rumen ciliates; but if the comparison was made at concentrations near saturation in the buffer employed, indole being the more soluble compound, the respective effects upon the final appearance of the cells were almost equally spectacular. Briefly, not only did all ciliary motion cease in two hours at 37°, but also a phenomenon resembling a drastic mechanical disintegration took place. The outer pellicles of the holotrichs, and also of *Ophryoscolex*, were torn asunder with liberation of the internal contents of the cells, so that after gently shaking the tube, instead of the rapid re-formation of a layer of ciliates at the bottom below a clear supernatant, there was a dense uniform turbidity throughout due to the release of storage polysaccharide granules and other inclusions from the disrupted cells.

The effect is markedly similar to that due to the anionic detergent 'Teepol', as described by Oxford<sup>3</sup>; but since indole and skatole do not, of course, greatly lower the surface tension of the buffer, another explanation of their action has to be found. Any such explanation must take account of the following facts:

(1) The rapid disruptive effect is only exhibited near the saturation point, that is, c. 0.1 per cent for indole, and 0.025 per cent for skatole (c. 0.002 *M*) in the buffer employed. This applies also to those isomers of skatole which have so far been tested, namely, 2- and 7-methylindoles. These appear to have similar solubilities to skatole, which, however, acts much more rapidly than either of the others and produces a denser final turbidity.

(2) Isatin, which is also sparingly soluble in the buffer (c. 0.12 per cent), acts in a fashion very similar to indole. Aniline, however, is not highly toxic.

(3) More highly polar indole derivatives related to tryptophan, like tryptamine, tryptophanol and indole-3-acetic, -propionic and -butyric acids, are not highly toxic and have no disintegrative effect at pH 7 at a concentration of 0.1 per cent.

(4) The toxic effect of indole or skatole cannot be nullified by addition of tryptophan, serine, casein hydrolysate or glucose to the buffer.

It would appear that although a fairly close resemblance to the tryptophan structure, as in skatole, increases the disruptive power of these indole derivatives, the primary requisites for toxicity are physical, namely, slight water- and ready lipoid-solubility, and the absence of any strongly polar centre in the molecule. Since indole—and perhaps skatole—are possible products of bacterial degradation of protein in the rumen, the presence of these and similar compounds may provide an explanation for some of the puzzling variations in density and composition of ciliate population in the normal rumen which undoubtedly occur from time to time.

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<sup>1</sup> Hogg, J. F., and Elliott, A. M., *J. Biol. Chem.*, **192**, 131 (1951).

<sup>2</sup> Heald, P. J., Oxford, A. E., and Sugden, B., *Nature*, **169**, 1055 (1952).

<sup>3</sup> Oxford, A. E., *J. Gen. Microbiol.*, **5**, 83 (1951).

### Goitre and the Iodine Content of Cow's Milk

DURING a recent systematic investigation in the Netherlands, it was found that at all seasons marked differences existed between the iodine contents of milk supplied to creameries in goitrous and non-goitrous areas. The location of these areas, in general, corresponds with those found by investigations on thyroid enlargement and thyroid histology in humans and farm animals<sup>1</sup>.

In addition to the local differences, a seasonal variation, roughly corresponding inversely to the milk yield, was observed. Therefore, it is essential to make the period of collection of the milk samples as short as possible in order to get strictly comparable results. In the present investigation, some hundreds of samples were collected each time on the same day. The variation of iodine content with time has often been disregarded in previous investigations, and as a result the distinction of goitrous from non-goitrous regions by examination of the milk supply has been needlessly obscured.

In Table 1 a summary of the iodine values, obtained with our method of analysis<sup>2</sup>, is recorded.

For a general survey of the iodine status of cattle, the determination of iodine in the milk offers distinct advantages over determination in the blood or histological investigation of thyroid glands. Collection of milk samples is comparatively easy, and the large number of contributing animals living in the same conditions rules out factors of individual variation in quite a simple way.