partial desiccation, is an excellent tool for investigating the relationship between 'structural' water and the cell or virus and there can be no doubt that 99·9 per cent of these microbes die as a result of an irreversible change in the structure of their nucleic acids due to the loss of water molecules.

S. J. Webb

Department of Bacteriology,
University of Saskatchewan,
Saskatoon.

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Carbohydrase Activity of Rumen *Entodinium* Species from Sheep on a Starch-free Diet

Oligotrich ciliates from the rumen ingest plant particulate matter but, unlike the holotrichs, are apparently unable to utilize soluble sugars[1]. Some oligotrichs are able to utilize a wide range of plant carbohydrates; thus cell-extracts of *Epidinium ecaudatum*, isolated from cows fed fresh red clover, have been shown to contain amylase, maltase, hemicellulase[2] (xylanase plus arabinosidase), α-galactosidase[3] and pectinase[4]. By contrast one oligotrich species, *Entodinium caudatum*, appears to live solely on starch grains[5]. This was confirmed by Abou-Akkada and Howard[6], who found amylase and maltase, but no other carbohydrase, in cell-extracts of this organism prepared from a sheep fed on hay plus grain.

In connexion with these latter observations we were interested to observe that in the protozoa-rich rumen contents of sheep grazing pure rye-grass pastures nearly 80 per cent of the protozoa were *Entodinium* species. The entodinia were mainly *E. ovinum*, *E. longinucleatum*, *E. indicum*, *E. biconcavum* and *E. bicarinatum*; *E. caudatum* was absent. As rye-grass contains less than 0·7 per cent (dry wt.) of starch[7] it is, compared with clover or grain, a low starch diet suggesting that the large numbers of entodinia present were not predominantly plant starch eaters or obtained starch from some other source. Because of their abundance and relatively small size it proved possible to isolate, by differential centrifugation, an entodinia preparation free from other protozoa and relatively free from bacteria. Cell-extracts prepared from these *Entodinia* were tested for carbohydrase activity.

The entodinia were disrupted by treatment for 2 h in indole-buffer[8], which does not disrupt bacteria, and centrifuged to give extract *A* (25 ml.), after which the residue was re-extracted, by grinding with 'Ballotini' beads in citrate buffer (0·1 M, *p*H 6·0), and centrifuged to give extract *B* (25 ml.). Both extracts were re-centrifuged (25,000*g*) and dialysed at 2° against distilled water and citrate buffer respectively. The extracts were tested for carbohydrase activity in digests containing carbohydrate (5 mg), extract (0·5 ml.) and citrate buffer (0·5 ml., 0·1 M, *p*H 6·0) which were incubated at 37° for 24 h and analysed, by paper chromatography, for liberated sugars. Appropriate controls were included.

Extract *A* rapidly hydrolysed amylose and amylopectin (to maltose and maltotriose), xylan and arabinoxylan (to arabinose, xylose and xylobiose), laminarin (to glucose) and cellodextrins (to cellobiose and cellotriose). Maltose, laminaribiose and sucrose were hydrolysed at a much slower rate, while cellobiose, β-glucosides, α- and β-galactosides, pectin and native cellulose were not hydrolysed. Extract *B* contained weaker amylase, xylanase and cellodextrinase activity than extract *A* but hydrolysed maltose and laminaribiose at a faster rate and also hydrolysed cellobiose. Chromatograms of the xylan digests after only 6-h incubation showed the presence of a series of homologous oligosaccharides indicative of random cleavage of the xylan chain. Similar chromatograms of the cellodextrin digests suggested that in this case hydrolysis was by the endwise removal of cellobiose units. In contrast to the foregoing action of the entodinial xylanase, epidinial xylanase has been observed to hydrolyse xylan chains by the endwise removal of xylobiose units[2].

Entodinia have been reported[9] to ingest rumen bacteria the disruption, by digestion or autolysis, of which could provide bacterial starch. Neither of our extracts liberated any sugars from freeze-dried rumen bacteria although simple disruption of the bacterial cells should have exposed the intracellular starch to amylase activity. It is possible, however, that the ingested bacteria autolyse inside the living protozoa or that enzymes responsible for their disruption are present but were not extracted by our procedure. This latter possibility may also explain the absence of a true cellulase in our extracts.

The carbohydrase activity of the cell-extracts suggests that the mixed *Entodinia* from our grass-fed sheep are able to utilize carbohydrates other than starch and possibly even cellulose. In cultures in this laboratory some of the larger *Entodinia* have in fact been observed ingesting both cellulose and grass particles. The results suggest that so far as carbohydrate digestion is concerned the starch-eating *Entodinium caudatum* may be an atypical member of the genus *Entodinium*.

R. W. Bailey
R. T. J. Clarke

Plant Chemistry Division,
Department of Scientific and Industrial Research,
Palmerston North,
New Zealand.

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Effect of Carcinogenic Polynuclear Hydrocarbons on the Metabolism of Œstrogens

Huggins[1] in 1961 showed that a single feeding of certain polynuclear aromatic hydrocarbons rapidly evoked mammary tumours in female rats and that the incidence of cancer largely depended on the hormonal status of the animals. Furthermore, Dao[2] has demonstrated the failure of 3-methylcholanthrene to induce mammary cancer in male rats irrespective of the dose given and despite the fact that its concentration was the same in the mammary tissues of both sexes. Since, in addition, Conney, Miller *et al.*[3–6] have shown that these carcinogens rapidly induce the formation of hydroxylating and other enzymes in rat liver, it was decided to investigate the fate of orally administered polynuclear hydrocarbons on the metabolism of steroid œstrogens in hepatic tissue.

(16-^{14}C) œstrone or œstradiol ($3·7 \times 10^5$ c.p.m. in 10 μg) was incubated under oxygen for 30 min at 37° C with the supernatant fraction (8,000*g*) of a rat liver homogenate, or, occasionally, with the isolated (100,000*g*) microsomes, and the amount of radioactivity remaining in the aqueous medium after extraction with ether at *p*H 1 determined. In addition, each ethereal fraction was examined for œstrogen metabolites by chromatography in the toluene-propylene glycol[7] or a modified Bush[8] system (benzene, ethyl acetate, methanol, water, 19 : 1 : 4 : 16) followed by autoradiography[9]. The polynuclear hydrocarbons, dissolved in oil by gentle heating, were administered by