enzymes may prevent diffusion of the peptides into surrounding tissue, and serve to limit them to the particles in which they occur. The enzyme destroying substance P may possibly serve to terminate its action if substance P does act as a transmitting agent (Lembeck, 1953). Finally, the enzymes may assist in the maintenance of the amino acid pool in the hypothalamus, and the destruction of the four peptides may be incidental.

SUMMARY

1. Incubation of bradykinin, oxytocin, substance P and vasopressin with homogenate of dog hypothalamus at pH 7.3 and 37° caused a reduction in the biological activity of the polypeptides.

2. The inactivation of the polypeptides is due to enzymes; bradykinin, oxytocin and vasopressin are destroyed most rapidly at pH 7-8, and substance P at pH 6.

3. The general properties of the enzyme destroying vasopressin, taken in conjunction with the effect of some enzyme inhibitors, suggest that it is different from enzymes previously found in brain.

4. Results obtained with mixed substrates suggest that oxytocin and vasopressin are substrates for the same enzyme; substance P appears to be inactivated by a different enzyme.

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Carbohydrases of the Rumen Ciliate *Epidinium ecaudatum* (Crawley)

ACTION ON PLANT HEMICELLULOSE

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The fermentation in the rumen of the hemicellulose fraction of pasture plants is well established. Several species of rumen bacteria, notably a *Butyrivibrio* species (probably *Butyrivibrio fibrisolvens*) (Howard, Jones & Purdom, 1960) and *Bacteroides amylogenes* (Howard *et al.* 1960), can

utilize the xylan which is a major constituent of hemicelluloses. So far as we are aware, however, no species of rumen ciliate protozoa has been reported to hydrolyse or ferment this type of polysaccharide. Four species of rumen ciliates, *Dasytricha ruminantium*, *Isotricha prostoma*, *Isotricha*

intestinalis and *Entodinium caudatum*, have actually been shown, *in vitro*, to be incapable of either hydrolysing xylan or of fermenting free pentoses (Howard, 1959; Abou-Akkada & Howard, 1960).

At this Laboratory it is comparatively easy to obtain virtually pure bacteria-free preparations of the oligotrich ciliate *Epidinium ecaudatum* (Crawley) from the rumens of cattle feeding on fresh red clover (*Trifolium pratense*, L.). Initial cultural and biochemical studies (Bailey, 1958; Oxford, 1958, 1959) suggested that this organism, like *Entodinium* species, consumed primarily starch so far as its carbohydrate diet was concerned. In recent studies, however, Wright (1961) found that *Epidinium* could produce gas from depectinated clover fibre, but not from the same fibre extracted with dilute alkali. A possible explanation of these results was that the protozoa were fermenting at least part of the hemicellulose fraction of the fibre, presumably after hydrolysis by hemicellulase action.

The presence of hemicellulase activity in *Epidinium ecaudatum* would seem to be of considerable interest in view of its absence from other major genera of rumen ciliates. As part of a programme on the carbohydrate metabolism of rumen protozoa an investigation of the action of cell-free extracts of the organism on plant hemicelluloses was undertaken and the results obtained form the subject of this paper.

MATERIALS AND METHODS

Carbohydrates

Mono- and di-saccharides. Except where stated otherwise, these were from the usual commercial sources and were shown, by paper chromatography, to contain not more than traces (less than 0.5% by visual estimation) of contaminant sugars. Commercial maltose was purified by adsorption on charcoal-Celite followed by fractional elution with aq. ethanol. Xylobiose (4-O- β -D-xylopyranosyl-D-xylose) was isolated, by aq.-ethanol fractionation on charcoal-Celite, from a partial acid hydrolysate (0.1N-H₂SO₄ at 100° for 1 hr.) of xylan obtained from Mann Research Laboratories Inc., New York.

Hemicellulose. The following hemicellulose fractions were used.

Wheat-flour xylan was isolated from unbleached wheat flour by the method of Howard (1957).

Clover hemicellulose was isolated by the following procedure. Freshly cut red-clover stems and petioles were macerated in a blender in aq. 80% (v/v) ethanol and extracted successively with boiling aq. ethanol, boiling water and hot (80–85°) 0.5% ammonium oxalate to remove sugars, pectin and other soluble polysaccharides. The fibrous residue was dried in an air oven, milled to a fine powder and extracted for 30 min. at room temperature, under nitrogen, with aq. KOH [17%, w/v; 100 ml./10 g. of fibre (Waite & Gorrod, 1959)]. Undissolved fibre was removed by centrifuging and the total hemicellulose fraction precipitated with ethanol (2 vol.) after acidification to

pH 3.5 with concentrated HCl. The flocculent precipitate was centrifuged, suspended in water, dialysed against tap water for 2 days and finally freeze-dried. The yield represented 10% (on a dry wt. basis) of the original clover.

Wheat-straw hemicellulose was isolated from clean chopped wheat straw by the procedure used for preparing clover-stem hemicellulose.

Portions (10 mg., corrected for moisture) of each hemicellulose were hydrolysed in N-H₂SO₄ (2 ml.) for 3 hr. at 100° and the hydrolysates analysed for total reducing sugars. The wheat-flour, clover and wheat-straw hemicelluloses yielded 95, 85 and 70% of reducing sugars, calculated as anhydro-xylose, respectively. The hydrolysates of the first two hemicelluloses were desalted with Bio-Deminolit (CO₂ form) (The Permutit Co. Ltd.), and the concentrations of the individual monosaccharides were measured by quantitative paper chromatography. The wheat-flour xylan contained xylose (80%), arabinose (13%) and glucose (1%) but no other sugars. The clover hemicellulose contained xylose (70%), arabinose (3%), glucose (7.8%), galactose (3.2%) and a uronic acid (3.0%); wheat-straw hemicellulose contained the same constituent sugars in approximately the same proportions. The commercial xylan (Mann Research Laboratories Inc.) yielded on hydrolysis xylose and a trace of arabinose.

All three preparations and the commercial xylan were used in qualitative tests for hemicellulase activity. The wheat-flour xylan was used in all quantitative work as it was the only preparation which was completely water-soluble.

Preparations of protozoa

Rumen liquor was removed from a fistulated cow 10–30 min. after feeding on fresh red clover and the ciliate protozoa were separated by the method of Oxford (1958). The protozoa so obtained were freed from most of the external bacteria by repeated washing with Coleman's (1958) buffer and used for the preparation of *Epidinium ecaudatum* or mixed holotrichs as follows.

Epidinium ecaudatum. The mixed protozoal preparation was incubated overnight at 39° in Coleman's buffer containing potassium benzylpenicillin (0.004%) and neomycin sulphate (0.003%) to arrest bacterial action and mannose (0.5%) to kill the holotrichs (Sugden & Oxford, 1952). Active epidinia were recovered after incubation by repeated suspension and centrifuging, in fresh buffer containing the antibiotics, to remove holotrich debris and any dead bacteria.

Holotrichs. The mixed protozoa were incubated overnight at 39° in Coleman's buffer without mannose or antibiotics. They were then washed free of bacteria and dead oligotrichs by repeated suspension and centrifuging.

All protozoal preparations were checked microscopically for purity (as regards protozoa) and, when not required immediately, were stored at –20°. Microscopic examination of the epidinia preparations showed the presence of less than 1% of other protozoa which included a few intact, but apparently dead, holotrichs.

Preparations of rumen bacteria

Freshly collected rumen liquor (300 ml.) was diluted with an equal volume of Coleman's buffer and centrifuged three times for 15 min. (500, 500 and 1000g) to remove protozoa, plant debris and large bacteria. The bulk of the rumen bacteria were finally harvested by centrifuging at

25 000g. Microscopic examination showed that they were apparently free from protozoa. Water extracts were prepared from the bacteria by both of the mechanical techniques used for disrupting protozoa (see below).

Protozoal extracts

Epidinium ecaudatum. The extraction procedure used by Bailey (1958) was employed. Cells (4–6 g. wet wt.) in water (10 ml.) were disrupted by maceration in a Teflon tissue homogenizer for 25–30 min. at 0°, diluted to 20 ml. with water and centrifuged for 30 min. at 0° and 25 000g to give extract A. The cell debris was then successively extracted in the homogenizer for 5 min. at 0° with water and phosphate buffers. Each extract was centrifuged twice (25 000g) to ensure removal of debris and bacterial cells, diluted to 20 ml. and stored at 2° under toluene. Five successive extracts were obtained: A and B with water, C with dilute phosphate buffer (KH_2PO_4 , 3 mM; Na_2HPO_4 , 3 mM; NaCl, 20 mM; MgSO_4 , 1 mM; pH 7.0), and D and E with more concentrated phosphate buffer (as for C but with concentrations 10 times greater).

The five extracts were prepared from three samples of *epidinia* (EI, EII and EIII; 5, 6 and 10 g. wet wt. respectively) harvested from rumen contents collected on widely separate occasions (2–3 months). Extracts EI–A and EII–A, used in the quantitative experiments, contained 0.42 and 0.58 mg. of nitrogen/ml. respectively. *Epidinia* sample EIII was halved; one half (EIV) was disrupted and extracted in the normal manner and the second half (EV) was disrupted in water by the grinding method used for holotrichs (see below) and then extracted into the usual five extracts (each 5 min. at 0° in the Teflon homogenizer).

Holotrichs. Mixed holotrichs (6–10 g. wet wt.) were mixed with Ballotini spheres (no. 14) and a little acetate buffer (0.05M, pH 6.7) to form a thick paste. The mixture was cooled to 0° and gently ground for 4–5 min. with a mortar and pestle, disrupting 90% of the protozoa. Additional buffer (10 ml.) was added to the paste and, after grinding for a further 2 min., the suspension was centrifuged at 0° and 25 000g. The supernatant was centrifuged again, diluted to 20 ml. and stored at 2° under toluene.

Sugar analyses

Reducing sugars. These were measured in hydrolysates by the cuprimetric method of Shaffer & Hartmann (1921) and calculated as xylose or glucose from appropriate standard curves.

Paper chromatography. For qualitative analyses of hydrolysates and the identification of sugars the solvents and sprays detailed by Bailey & Robertson (1962) were used. Quantitative paper-chromatographic analyses were done by streaking measured volumes of hydrolysates, plus marker spots, on sheets of Whatman no. 1 paper. After development the sugars were eluted from the appropriate strips and measured by the sulphuric acid method of Bath (1958).

RESULTS

Action of protozoal extracts on carbohydrates

Hydrolysis of various carbohydrates by extracts of epidinia. Previous work (Bailey, 1958) had shown that the water extracts (A and B) were rich in

amylase activity and the more concentrated buffer extracts (D and E) contained mainly maltase activity. The five extracts from each *epidinia* preparation were, therefore, tested against wheat-flour xylan, xylobiose, starch and maltose in standard solutions containing extract (0.5 ml.), carbohydrate (10 mg.) and acetate buffer (0.05M, pH 6.0, 0.5 ml.). The digests were incubated for 24 hr. at 39° and analysed by paper chromatography both before and after incubation. The chromatograms indicated that, in each set of *epidinia* extracts, the two water extracts (A and B) were rich in hemicellulase and amylase and also contained xylobiase but no maltase. The dilute buffer extract (C) contained very little carbohydrate activity, and the more concentrated buffer extracts (D and E) contained maltase activity but did not hydrolyse the other carbohydrates. Extracts D and E were reserved for a study of the *epidinia* maltase. Control digests lacking either the protozoal extracts or added carbohydrates were also incubated, but the chromatograms showed no sign of any sugar not added. Extracts A were tested against additional sugars in standard solutions incubated and analysed as described. Cellobiose, gentiobiose, salicin, melibiose, isomaltose and sucrose were hydrolysed to varying extents; powdered cellulose, dextran, α -trehalose, lactose, turanose, and methyl α -D-glucose were unattacked.

The rates of hydrolysis of the various carbohydrates were compared in digests containing extract (EIII–A, 0.1 ml.; calculated to hydrolyse approximately half of the wheat-flour-xylan links in 24 hr.), acetate buffer (0.05M, pH 6.0, 2.9 ml.) and carbohydrate (10 mg.). The digests were incubated for 24 hr. at 39° and reducing sugars measured before and after incubation (Table 1).

Hydrolysis of hemicellulose fractions by extracts of epidinia. Standard solutions containing extract (EI–A) and one each of the three hemicellulose preparations and commercial xylan were incubated and analysed by paper chromatography as described above. After incubation strong monosaccharide spots were detected on chromatograms of all four solutions. The main monosaccharide spot in all four solutions was chromatographically identical with xylose. Other spots present were shown to be identical with the following sugars. Wheat-flour xylan: arabinose, glucose (trace); commercial xylan: arabinose (trace); clover hemicellulose: arabinose, glucose, galactose and uronic acid; wheat-straw hemicellulose: arabinose, glucose, galactose and uronic acid. The order of intensity of the spots was similar to that found in the acid hydrolysates. The same results were obtained when extracts A and B from EI, EII, EIV and EV were incubated with each of the hemicelluloses.

Action extracts of holotrichs and of rumen-bacteria on hemicelluloses. Standard solutions containing holotrich extract and hemicellulose were incubated and analysed by the method used for the epidinial extracts. Paper-chromatographic analyses showed that the extracts were without action on any of the hemicelluloses. Under the same conditions the extracts were without action on melibiose but rapidly hydrolysed sucrose. Extracts from mixed rumen bacteria, prepared by both mechanical disruption techniques, were incubated with the hemicellulose fractions under the standard conditions. Paper-chromatographic analyses showed that both extracts were without action on the hemicelluloses.

A portion of the rumen bacteria was incubated overnight at 39° in Coleman's buffer containing the antibiotics. The suspension was centrifuged at 25 000g, the supernatant preserved, and the solid residue extracted in the Teflon tissue homogenizer, as for the epidinia, to yield a water extract. The water extract and the supernatant from the incubation were tested against wheat-flour xylan and amylose under the standard conditions and found, by paper-chromatographic analysis, to have no action on either polysaccharide.

Release of carbohydrases from protozoa by indole. Preparations of mixed rumen protozoa (4–6 g. wet wt., approximately one-third *Epidinium caudatum*), freed as far as possible from external rumen bacteria, were suspended in Coleman's buffer (20 ml.) containing indole [0.1% (Eadie & Oxford, 1954)] but no antibiotics and were incubated at

39° for 2 hr. After this time, microscopic examination showed that virtually all of the protozoa had disintegrated. The extracts were centrifuged twice at 25 000g to remove debris and any bacteria. Solutions were prepared containing extract (0.1 ml.), acetate buffer (0.05M, pH 6.0, 0.3 ml.) and carbohydrate (10 mg.), incubated for 24 hr. at 39° and analysed by paper chromatography before and after incubation. The chromatograms showed that the extracts readily hydrolysed wheat-flour xylan, amylose, maltose, salicin and sucrose; other sugars were not tested. A preparation of the epidinia (1–2 g. wet wt.) was similarly treated with indole and was completely disintegrated by the indole after incubation for 3 hr. When tested under the standard conditions, this cell-free epidinial extract obtained readily hydrolysed hemicellulose and amylose but scarcely attacked maltose. When protozoa-free preparations of rumen bacteria were treated with indole under the same conditions, the cell-free extracts obtained did not attack wheat-flour xylan, amylose, maltose or salicin, but did hydrolyse sucrose very slowly.

Effect of various conditions on the action of epidinial hemicellulase

Rate of hydrolysis of hemicellulose and xylobiose. A solution containing epidinial extract (EI-A, 0.9 ml.), acetate buffer (0.1M, pH 6.0, 8.1 ml.) and wheat-flour xylan (45 mg.) was incubated at 39°, and at intervals portions were removed for reducing-sugar and chromatographic analyses. The reducing-sugar analyses plotted against time of incubation are given in Fig. 1.

During the first 2 hr. of incubation, spots were detected on the chromatograms corresponding to

Table 1. *Action of epidinial extract on various carbohydrates*

Solutions containing extract (EIII-A, 0.1 ml.), carbohydrate (10 mg.) and acetate buffer (0.05M, pH 6.0, 2.9 ml.) were incubated at 39° for 24 hr. Reducing sugars were measured before and after incubation and calculated as the appropriate monosaccharide, except for the amylose solution when they were calculated as maltose.

Added carbohydrate	Increase in reducing sugars (mg./ml.)	Hydrolysis of glycosidic links (%)
Wheat-flour xylan	1.90*	55.8
Amylose	2.64	71.3†
Xylobiose	0.50	14.7
Cellobiose	0.72	21.1
Sucrose	0.80	23.2
Isomaltose	0.40	11.7
Melibiose	0.32	9.2
Salicin	0.20	9.0
Gentiobiose	0.28	8.2
Maltose	0	0

* Corrected for xylobiose.

† Approximate only; chromatograms showed in addition to maltose detectable amounts of glucose, maltotriose and higher maltodextrins.

Fig. 1. Effect of anions on the rate of hydrolysis of wheat-flour xylan by epidinial hemicellulase. Solutions containing epidinial extract (EI-A, 0.9 ml.), buffer (0.1M, pH 6.0, 8.1 ml.) and wheat-flour xylan (45 mg.) were incubated at 39° and reducing sugars measured at intervals. ▲, Citrate buffer; ●, phosphate or acetate buffer.

Table 2. *Rate of liberation of reducing sugars from wheat-flour xylan by epidinial extract*

Solution containing extract (EIII-A, 0.6 ml.), wheat-flour xylan (60 mg.) and acetate buffer (0.05 M, pH 6.0, 8.4 ml.) was incubated at 39°. Portions were analysed at intervals for individual sugars by quantitative paper chromatography.

Time of incubation (hr.) ...	Reducing sugar (mg./ml.)					
	0	1	2	4	6	24
Xylose	—	—	Trace	0.30	0.40	1.30
Arabinose	—	0.10	0.30	0.37	0.50	0.92
Xylobiose	—	Trace	0.20	0.40	0.50	0.90

arabinose (intense spot), xylose (weak spot) and xylobiose (moderately intense spot). As the incubation proceeded, the arabinose spot remained of constant intensity, but the xylobiose spot at first increased in intensity (2–6 hr.) and then gradually disappeared as xylose accumulated. The disappearance of xylobiose, however, did not always occur (see Table 2). When the experiment was repeated with clover-stem or wheat-straw hemicellulose, similar results were obtained with a rapid release of arabinose followed by xylobiose and xylose; the glucose, galactose and uronic acid components appeared only after incubation for several hours. At no stage was there any sign of components corresponding to xylotriose or higher homologues.

Solutions were prepared containing epidinial extract (EIII-A, 0.6 ml.), acetate buffer (0.05 M, pH 6.0, 8.4 ml.) and wheat-flour xylan (60 mg.) and incubated for 24 hr. at 39°; during this time approximately half of the xylan links were hydrolysed. At intervals portions (1 ml.) were removed from the digests, heated for 5 min. at 100°, and analysed by quantitative paper chromatography for arabinose, xylose and xylobiose (Table 2).

When this experiment was repeated with commercial xylan containing only a trace of arabinose, xylobiose (0.2–0.3 mg./ml. of solution) was present after only 1 hr. of incubation.

A solution containing extract (EI-A, 0.3 ml.), acetate buffer (0.1 M, pH 6.0, 2.7 ml.) and xylobiose (15 mg.) was also incubated at 39° and analysed for reducing sugars at intervals. The increase in reducing power after incubation for 7 hr. corresponded to a release of 0.76 mg. of xylose/ml. of solution; during this time the release of reducing sugars, as xylose, from the same amount of wheat-flour xylan was 2.16 mg./ml. of solution. Similar results were obtained with extract EII-A and EIII-A (see Table 1).

Effect of anions on hemicellulase activity. The rate-of-hydrolysis experiment with wheat-flour xylan described above was repeated except that the buffer used was phosphate or citrate (0.1 M, pH 6.0). Reducing sugars were measured at intervals during the incubation (Fig. 1). Citrate had an apparent depressing effect on the rate of hydrolysis, but phosphate and acetate gave the same results and

Fig. 2. Effect of pH on hemicellulase action. Solutions containing epidinial extract (EI-A, 0.3 ml.), buffer (0.1 M; acetate, pH 4.0–6.0, or phosphate, pH 6.0–8.0; 2.7 ml.) and wheat-flour xylan (15 mg.) were incubated at 39°. Reducing sugars were measured after incubation for 3 hr. (▲) and 7 hr. (●).

these two buffers were used in investigating the effect of pH on enzyme activity.

Effect of pH on hemicellulase and xylobiase activity. Solutions containing epidinial extract (EI-A or EII-A, 0.3 ml.), buffer (0.1 M; acetate, pH 4.0–6.0, or phosphate, pH 6.0–8.5; 2.7 ml.) and wheat-flour xylan (15 mg.) were incubated at 39°, and reducing sugars were measured at intervals. The results obtained, plotted against pH, after incubation for 3 and 7 hr. are shown in Fig. 2.

Similar solutions containing extract (EI-A or EII-A, 0.1 ml.), acetate or phosphate buffer (0.1 M, pH 5.0–7.5, 0.3 ml.) and xylobiose (3 mg.)

were incubated at 39° and analysed at intervals by paper chromatography. Xylobiase activity at various pH values was assessed by comparing the intensity of the xylose spots on the papers, and was clearly optimum in the pH range 5.8–6.2 and very much lower in the range 6.8–7.5.

Effect of temperature on hemicellulase activity. Solutions containing epidinial extract (EI-A or EII-A, 0.3 ml.), acetate buffer (0.1 M, pH 6.0, 2.7 ml.) and wheat-flour xylan (15 mg.) were incubated at various temperatures (30–55°), and reducing sugars were measured at intervals. Activity increased with increasing temperature up to 38–40°, then remained almost constant up to 50°; above this temperature activity declined rapidly. In the temperature range 38–50° the rate of activity during incubation for 8 hr. was reasonably constant after allowing for the initial rapid release of arabinose. The aqueous solutions of the enzymes retained full hemicellulase activity during storage for several months at 2° under toluene.

DISCUSSION

Until it is possible to grow rumen ciliates in truly bacteria-free cultures care must be taken to ensure that carbohydrases claimed to have been extracted from the ciliate preparations have not in fact come from contaminating bacteria. In the procedure for isolating epidinia in the present work the bulk of the external bacteria are removed in the preliminary washing. Sufficient bacteria remain, however, to make the use of antibiotics essential in the subsequent incubation for the removal of the holotrichs, otherwise the epidinia themselves are largely destroyed by the fall in pH. The antibiotic treatment has been shown by Oxford (1958) to kill all of the remaining external bacteria although some of the internal bacteria remain viable. The final washings to remove dead holotrichs also remove many of the dead external bacteria. Although most of the external bacteria are removed during processing there is still a remote possibility that the extracted hemicellulase had come from the residual bacteria, possibly after the antibiotics had rendered them more liable to disruption. However, neither of the techniques used for the mechanical disintegration of the epidinia extracted any hemicellulase from fresh rumen bacteria. If the enzyme had in fact come from residual bacteria it would probably also have been present in the holotrich extracts; these extracts were free from hemicellulase. In addition, treatment of mixed rumen bacteria with the antibiotics did not liberate hemicellulase or enable the enzyme to be extracted from them. The uncertainty as to the specificity for protozoa of the mechanical means of disintegration was largely removed by treating

both freshly isolated mixed rumen ciliates (about one-third of which were epidinia) and epidinia alone with indole. The protozoa were easily disrupted and hemicellulase and other carbohydrases liberated, but no hemicellulase was liberated from protozoa-free rumen bacteria by this treatment. The above evidence indicates that the hemicellulase in the epidinial extracts had come from the epidinia themselves and not from any residual bacteria.

The ease of extraction of the epidinial hemicellulase in water, once the cells have been disrupted, is in marked contrast with the insolubility of rumen-bacterial hemicellulase. Thus Howard *et al.* (1960) only obtained bacterial hemicellulase by the preparation of butan-1-ol-dried powders of the cells. As with the hemicellulase, an α -amylase (Bailey, 1958) is extracted from the epidinia by simple disruption in water, and this latter enzyme is no doubt important in the known fermentation of starch by epidinia (Oxford, 1958). Because of the different mixtures of sugars produced, it is not easy to compare directly the α -amylase and hemicellulase activities of the extracts. The results (Table 2) do suggest, however, that the rates of hydrolysis of amylose and hemicellulose are of the same order and that the hemicellulase is not a trace contaminant. The results obtained with epidinial hemicellulase and xylobiase suggest that they are not the same enzymes as those isolated from the *Butyrivibrio* and *Bacteroides amylogenes* by Howard *et al.* (1960); the hemicellulases of these latter organisms appeared to hydrolyse the xylan chain by a mechanism different from that of epidinial hemicellulase (see below). In addition, the pH optimum of the epidinial xylobiase (5.8–6.2) is clearly different from that reported by Howard *et al.* (1960) for the two bacterial xylobiases (6.8).

Some evidence regarding the mode of hydrolysis of wheat-flour xylan by the epidinial hemicellulase is given by the chromatographic analyses of the hydrolysates. The initial release of arabinose without much accompanying xylose or xylobiose suggests that the arabinose, largely present as branch units along the main xylan chains, is removed before the xylan itself is attacked. Howard (1957) observed a similar early release of arabinose from wheat-flour xylan by rumen bacteria. With the *Butyrivibrio* and *B. amylogenes* enzymes he suggested that a separate arabinosidase was responsible and that the presence of the arabinose units hindered hydrolysis of the xylan chain by a separate xylanase. Soon after the release of part of the arabinose by the epidinial enzyme, xylobiose was liberated in easily detectable amounts, no sign of xylotriose or any higher homologue being detected in the solutions at any stage during the incubation. These results suggest that

the xylan chain is hydrolysed by the successive removal of xylobiose units from one end of the chain and not by random cleavage. In contrast Howard *et al.* (1960) detected xylose oligosaccharides of a high degree of polymerization during all but the last stages of the hydrolysis, which was evidently by random cleavage of the chains.

In addition to hydrolysing the relatively simple xylans and arabino-xylans, *Epidinium* hemicellulase also hydrolysed the more complex mixture of polymers in clover or wheat-straw hemicellulose. It is not clear how sugars other than xylose and arabinose are released from these polymers; they were, however, detected only in the later stages of the hydrolysis. Probably the hydrolysis of these hemicellulose preparations by the epidinial extracts represents the action of a mixture of several enzymes, and the collective term 'hemicellulase' has been used throughout the present paper in view of this possibility. Presumably the xylobiase is a separate enzyme, although it might be a less specific β -1,4-glycosidase responsible also for the hydrolysis of cellobiose. The β -glucosidase and β -fructofuranosidase activity may have come from the few intact holotrichs remaining in the epidinial preparations.

So far as we are aware, the results reported in the present paper provide the first indication that at least one species of rumen ciliate, *Epidinium ecaudatum*, contains enzymes capable of hydrolysing plant hemicellulose and is thus able to contribute to the fermentation of this plant fraction in the rumen.

SUMMARY

1. Extracts prepared from the rumen protozoan, *Epidinium ecaudatum* (Crawley), under conditions which would not be expected to disrupt any

associated bacteria, readily hydrolysed various plant hemicelluloses and starch. Xylobiose, cellobiose, sucrose, isomaltose and melibiose were also hydrolysed, but not cellulose.

2. The hemicelluloses were hydrolysed to their constituent monosaccharides by a mechanism which involved the initial liberation of arabinose followed by the release of xylobiose and finally of xylose, glucose, galactose and uronic acid.

3. The hydrolysis appeared to involve cleavage of xylobiose from the end of the xylan chain rather than random cleavage of the chain.

4. The hemicellulase and the xylobiase both possessed optimum activity in the pH range 5.8–6.2, and the hemicellulase activity was almost constant over the temperature range 37–51°.

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The Metabolism of Isolated Rat-Kidney Nuclei

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Rees & Rowland (1961*a*) described the metabolism and chemical composition of isolated rat-liver nuclei. The nuclei incorporated amino acids into nuclear protein and adenine and orotic acid into ribonucleic acid. These processes were in-

hibited by anoxia, 2,4-dinitrophenol, chlorpromazine and azide. An enzyme system capable of oxidizing reduced diphosphopyridine nucleotide was detected and it was suggested that the liver nuclei possessed an oxidative energy-producing