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# INVESTIGATION OF THE DIGESTION OF CELL-WALL POLYSACCHARIDES OF SPEAR GRASS AND OF COTTON CELLULOSE BY VISCOMETRY AND BY X-RAY DIFFRACTION*

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ABSTRACT

The lag of ~10 hours in the onset of digestion of cotton cellulose in the rumen, observed by previous workers, has been confirmed. The molecular weight of the remaining cotton decreases only slowly during digestion, and the polysaccharide retains its fibrous form. The crystallinity decreases slightly at the same time, and it is concluded that the amorphous and crystalline regions of cellulose are attacked at approximately the same rate. The hemicelluloses of grass partly digested in the rumen and of faeces fibre have been isolated and found by viscometry to have molecular weights similar to those of the material isolated from the original grass. This finding confirms earlier conclusions that the digestion-resistant hemicelluloses are chemically identical with the digestible hemicelluloses and that the resistance is due to protection by lignin. The holocellulose prepared from faeces fibre by removal of lignin showed slightly less X-ray crystallinity than that from the original grass, but this effect is probably due to a decrease in cellulose–hemicellulose ratio during passage through the animal, rather than to preferential digestion of crystalline cellulose. A comparison of the chemical composition of the polysaccharides of grass and faeces fibre confirms that cellulose is digested more rapidly and completely than hemicelluloses, presumably because it is less effectively protected by lignin. In the corresponding holocelluloses, however, where the lignin has been removed, the cellulose and hemicelluloses are digested at about the same rate.

## INTRODUCTION

The digestion of cotton cellulose in the bovine rumen has been investigated by several workers. Grosskopf[1] and McBee[2] found that purified celluloses were digested only after a lag period of several hours after entering the rumen. Other workers[3–7] have shown that isolated celluloses are 90 to 100% digested by rumen micro-organisms, *e.g.*, cotton was 97% digested[3]. More recently, McManus *et al.*[8] showed

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that the dry-matter disappearance of cotton, using the Terylene bag technique, was ~97% after 72 h when sheep were fed a lucerne diet. It is well known that cotton fibres consist of almost pure cellulose which contains both crystalline and amorphous regions, but there appears to have been little previous work that would indicate whether or not the rumen micro-organisms may attack amorphous regions more rapidly than the crystalline regions. Baker *et al.*[9] had found an inverse relationship between the crystallinity (as measured by the height–width ratio of the major X-ray diffraction peak) and rate of digestion of cotton and isolated wood celluloses. However, subsequent work by Tomlin and Davis[10] revealed no definite relationship between crystallinity and digestibility.

It has been shown by several workers that delignified wood and herbage samples can be digested to a greater extent than the original lignified sample[5,7,11–14]. It is usually assumed that the indigestible lignin provides a physical barrier to the micro-organisms and is the major cause of incomplete digestion of cellulose and hemicelluloses. We were concerned, in the present studies, to determine the extent to which the crystallinity of the cellulose may also influence the rate of digestion of cell-wall polysaccharides of pastures.

## RESULTS AND DISCUSSION

Cotton samples, enclosed in Terylene bags, were digested in the bovine rumen over a period of 136 h, and the loss in weight during this time is shown in Table I. The characteristic lag period before digestion in the rumen began (up to 10 h in this case) has been detected by previous workers[1,2]. The lag may be associated with a requirement for the cellulolytic bacteria to become attached to, and perhaps grow viable colonies on, the fibre before dissolution commences.

TABLE I

D.P. AND RATE OF DIGESTION OF COTTON CELLULOSE

| *Raw cotton* | | | | | | *Commercial cotton* | | |
|---|---|---|---|---|---|---|---|---|
| In vivo | | | In vitro | | | In vivo | | |
| *Digestion time (h)* | *DMD*[a] (%) | *D.p.* | *Digestion time (h)* | *DMD*[a] (%) | *D.p.* | *Digestion time (h)* | *DMD*[a] (%) | *D.p.* |
| 0 | — | 3760 | 0 | — | 3760 | 0 | — | 800 |
| 3 | 1.0 | 3700 | 48 | 14.0 | 2950 | 3 | 1.0 | 823 |
| 9 | 3.0 | 3600 | 120 | 45.0 | 2500 | 9 | 1.0 | 880 |
| 24 | 12.5 | 3240 | 168 | 62.0 | 2800 | 24 | 10.3 | 995 |
| 40 | 43.6 | 2900 | 216 | 74.0 | 2540 | 48 | 38.1 | 1135 |
| 64 | 69.0 | 2540 | | | | 72 | 52.3 | 1070 |
| 88 | 81.0 | 2170 | | | | 96 | 65.5 | 1120 |
| 136 | 98.0 | 2700 | | | | 120 | 87.5 | 1350 |
| | | | | | | 144 | 95.5 | 1540 |

[a]Dry-matter digestion.

Table I shows that, for purified raw cotton, the d.p. of the undigested cellulose decreases with time. It is tempting to compare the digestion process with attack of dilute acid on cellulose, since such attack also occurs mainly in the amorphous regions. However, the acid attack results in a rapid decrease in d.p. of the undissolved residue to a "limit value" (~200) associated with the relatively acid-resistant hydrocellulose. In our case, the decrease in d.p. was a great deal less than that expected for preferential attack in the amorphous regions of the cotton fibre, indicating that the amorphous and crystalline regions are attacked at approximately the same rate. The physical appearance of the cotton during digestion confirmed this view, as the fibrous appearance was retained throughout the period investigated, although the fibres became mechanically weaker. This behaviour is quite distinct from a process (*e.g.*, acid hydrolysis) that causes dissolution of the amorphous regions only and converts the fibres into a powder. Careful examination of the cotton samples in the Terylene bags during the process of digestion showed no significant formation of a powdery product.

Table I also shows the results obtained in early experiments using commercial (bleached) cotton in order to illustrate a possible type of error in such studies. With the bleached cotton, there was an apparent *increase* in d.p. of the residue during progress of the digestion. This effect is considered to be misleading in terms of true digestion of the cellulose and the effect almost certainly arises from the presence of alkali-labile (oxidised) groups in the bleached cotton, which result in rapid chain scission when the sample is dissolved for viscometry. As digestion progresses, the proportion of alkali-labile groups in the residue decreases and hence the apparent d.p. increases.

Changes in crystallinity during digestion were investigated by X-ray diffractometry of the partly digested samples and compared with the original cotton. Qualitative, visual inspection of the resultant curves (Figs. 1 and 2) showed a slight decrease in crystallinity with digestion time. These results, taken together with data for dry-matter digestion, show that the amorphous and crystalline areas are digested at approximately the same rate.

The viscosity numbers of the hemicellulose fraction isolated from spear grass before and after digestion of the grass are shown in Table II. The difference in molecular size of the digestible and indigestible hemicellulose is evidentally very small. These results rule out any hypothesis whereby the hemicellulose molecule is attacked by exo-enzymes to decrease the chain length until the degradation of a particular molecule reaches a molecular heterogeneity, such as a branch point, and leaves a residue resistant to further digestion. We have previously shown[15] that the chemical differences between the original and the digestion-resistant hemicelluloses are also very small. The present results therefore provide further confirmation that the hemicellulose which resists digestion is similar to that which is digested, and thus confirm that the most likely cause of the resistance to digestion is physical protection by lignin.

The results of X-ray diffractometry on holocelluloses prepared from faeces

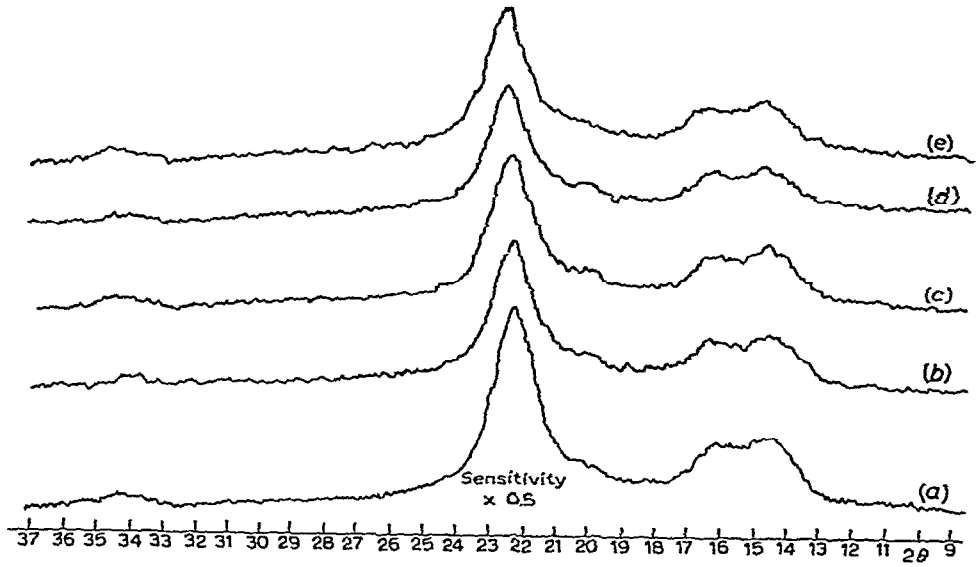


Fig. 1. X-Ray diffraction of cotton fibre during digestion in rumen: (*a*) original cotton; (*b*) 44%; (*c*) 64%; (*d*) 81%; (*e*) 98% digested.

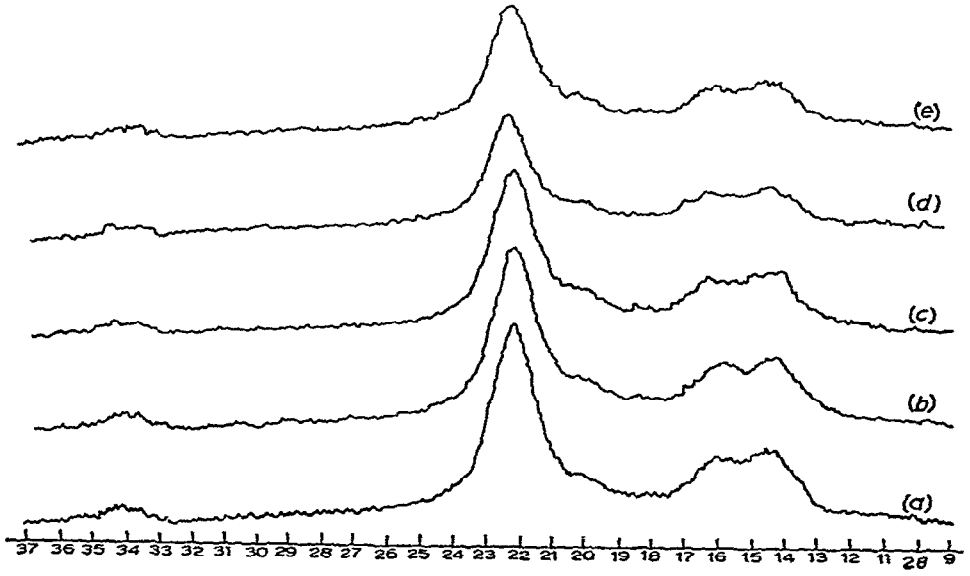


Fig. 2. X-Ray diffraction of cotton fibre *in vitro*: (*a*) original cotton; (*b*) 14%; (*c*) 28%; (*d*) 45%; (*e*) 74% digested.

TABLE II

VISCOSITY VALUES OF HEMICELLULOSES AFTER PARTIAL DIGESTION

| *Source of Hemicellulose B* | *Viscosity number* |
|---|---|
| Spear grass | 52.0 |
| Digested spear grass[a] | 50.5 |
| Faeces fibre | 49.8 |

[a]Three days in rumen.

fibre and from a preponderantly spear-grass pasture are shown in Fig. 3. These data confirm that there is less cellulose crystallinity in the faeces fibre than in the original grass, *i.e.*, the crystallinity decreases during the partial digestion. However, we have previously reported that the content of cellulose relative to hemicellulose in the holocellulose from faeces fibre is less than that from the corresponding spear-grass fodder[15], and this factor provides at least a partial basis for the decrease in X-ray crystallinity (which is due to the cellulose I lattice). It is possible also that the amorphous cellulose regions in the cell wall are more likely than the crystalline regions to be closely associated with hemicellulose and lignin, and hence are more protected from digestion. This effect would also contribute towards the observed decrease in crystallinity during digestion.

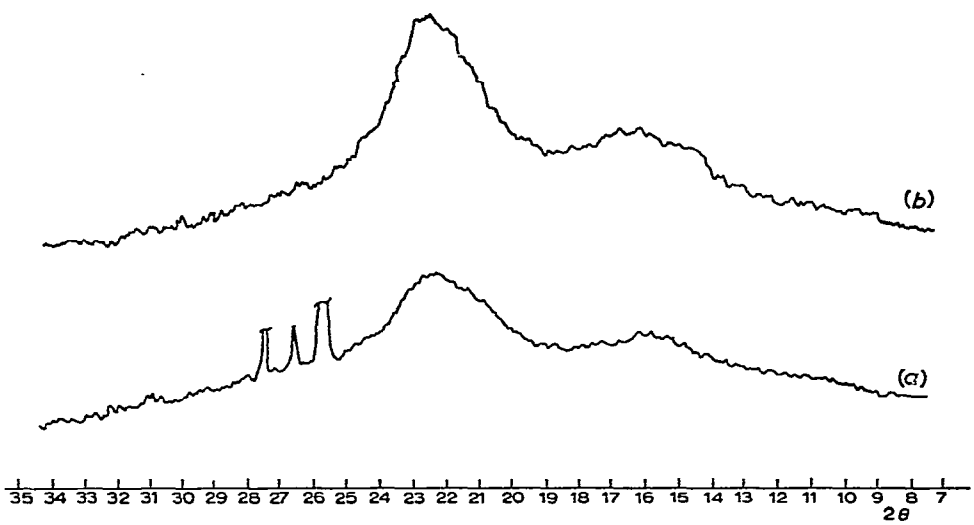


Fig. 3. X-Ray diffraction of holocelluloses: (*a*) from faeces fibre; (*b*) from spear grass.

In order to investigate further the digestion in the absence of lignin, two holocelluloses from spear grass and faeces fibre were subjected to digestion in Terylene bags. The dry-matter digestion of the faeces-fibre holocellulose after 89 h was 63%, compared with 13% before delignification (Table III). This finding is in

accordance with several previous reports that delignification brings about an increase in digestibility of grass fibre[5,7,11–14]. The results in Table III also show that hemicelluloses in the holocelluloses are rather more rapidly and completely digested than cellulose, and this is in contrast with the observations on the original grass before delignification[15]. Thus, the lignin appears to "protect" the hemicelluloses from digestion more effectively than it does the cellulose. We postulate that this effect is probably due to the proximity of regions of high lignin and high hemicellulose content in the fibre morphology and possibly also to the presence of hemicellulose–lignin linkages[16–21]. In both respects, the cellulose is likely to be less "protected" by lignin than are the hemicelluloses. Burdick and Sullivan[22] and Jarrige and Minson[23] have also reported experiments which suggest that, with increasing lignification of plants, the relative rate and extent of digestion of hemicelluloses decreases. The results of Okamoto and Hirose[24] on the digestion of delignified grass also confirm this effect.

TABLE III

CHANGES IN GLYCOSE COMPOSITIONAL ANALYSIS[24] OF FAECES-FIBRE AND SPEAR-GRASS HOLOCELLULOSES DURING FURTHER DIGESTION

| *Sample* | *Digestion time (h)* | | | | |
|---|---|---|---|---|---|
| | *0* | *17* | *41* | *65* | *89* |
| *Spear-grass holocellulose* | | | | | |
| DMD (%)[a] | 0 | 13.0 | 48.0 | 89.0 | 89.0 |
| Glucose | 65.0 | 66.5 | 66.5 | 67.0 | 67.5 |
| Xylose | 29.0 | 28.1 | 28.5 | 28.1 | 27.6 |
| Arabinose | 6.0 | 5.4 | 5.0 | 4.9 | 4.9 |
| Xylose+arabinose | 35.0 | 33.5 | 33.5 | 33.0 | 32.5 |
| Arabinose/xylose | 17/83 | 16/84 | 15/85 | 15/85 | 15/85 |
| *Faeces-fibre holocellulose* | | | | | |
| DMD (%) | 0 | 12.6 | 32.8 | 41.8 | 63.0 |
| Glucose | 50.0 | 52.0 | 54.0 | 56.0 | 54.0 |
| Xylose | 43.0 | 41.3 | 39.6 | 37.8 | 39.6 |
| Arabinose | 7.0 | 6.7 | 6.4 | 6.2 | 6.4 |
| Xylose+arabinose | 50.0 | 48.0 | 46.0 | 44.0 | 46.0 |
| Arabinose/xylose | 14/86 | 14/86 | 14/86 | 14/86 | 14/86 |
| *Faeces fibre* | | | | | |
| DMD (%) | — | 6.4 | 7.2 | 12.0 | 13.0 |

[a]Dry-matter digestion.

The X-ray diffraction patterns for the holocelluloses during progressive digestion are shown in Figs. 4 and 5. The extra peaks in Figs. 3 and 5 are probably due to silicates remaining in the faeces-fibre holocelluloses, which have high ash contents (Table IV). The X-ray diffraction shows no significant change in crystallinity with digestion of the holocelluloses. In the digestion of these materials, therefore, the hemicellulose and cellulose components disappear at approximately the same rate (Table III) as the amorphous and crystalline regions of cellulose (Figs. 1 and 2).

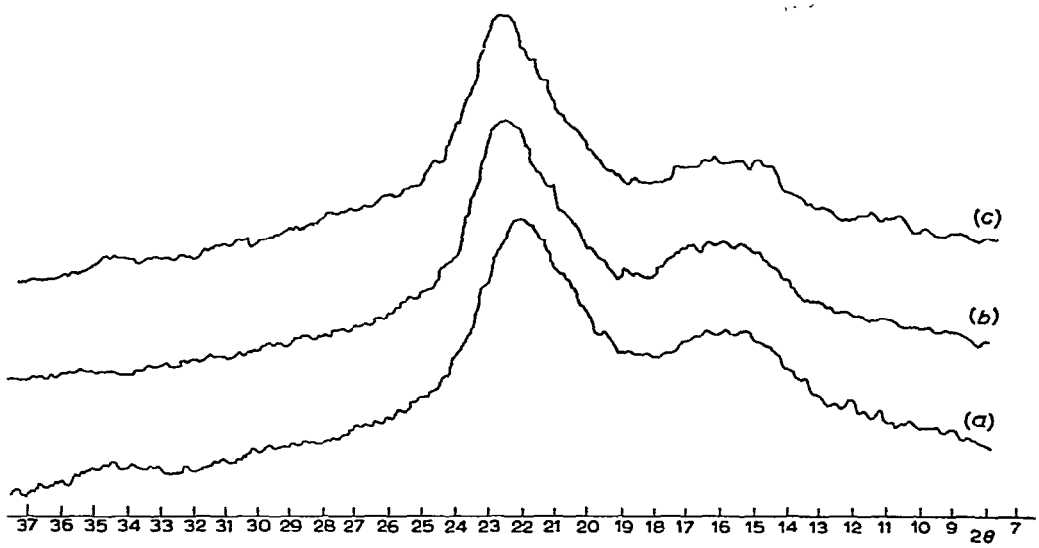


Fig. 4. X-Ray diffraction of spear-grass holocellulose during digestion in the rumen: (*a*) original holocellulose; (*b*) 48%; (*c*) 89% digested.

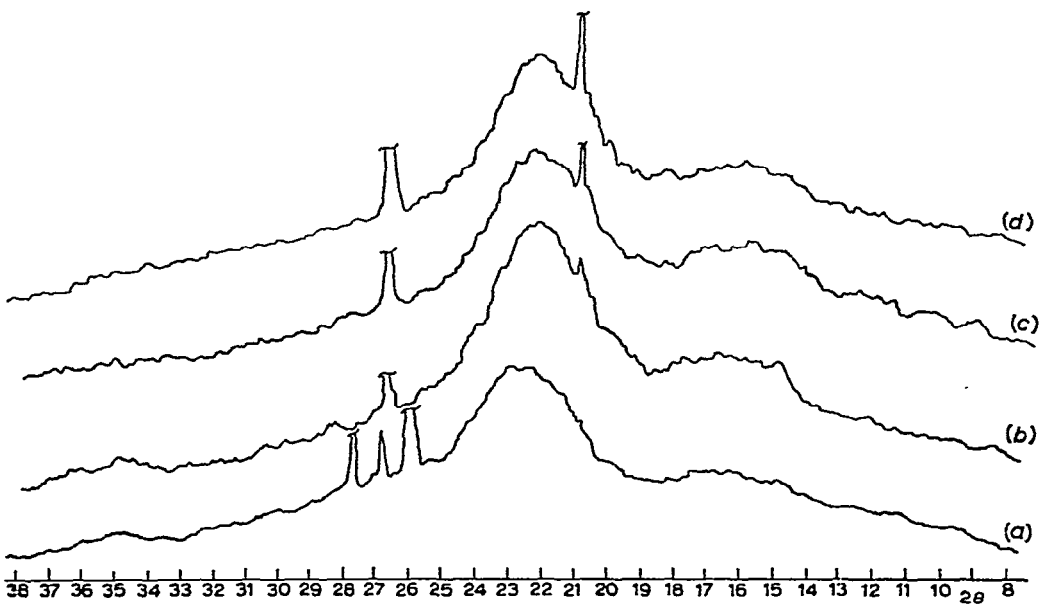


Fig. 5. X-Ray diffraction of holocellulose from faeces fibre during digestion in the rumen: (*a*) original holocellulose; (*b*) 33%; (*c*) 42%; (*d*) 63% digested.

TABLE IV

D.P. OF α-CELLULOSE FRACTIONS

| *Source* | *D.p.*[a] | *Material insoluble in cadoxen* (%) | *Ash* (%) |
|---|---|---|---|
| Spear grass | 2000 | 12.0 | 3.1 |
| Digested spear grass | 1940 | 17.0 | 4.0 |
| Faeces fibre | 1950 | 21.5 | 14.5 |

[a]Concentrations corrected for insoluble component.

The molecular weight of α-cellulose from several feeds, and from faeces fibre, has been determined for comparison with results from cotton. Table IV shows that the d.p. of residual α-cellulose after digestion of grass is about the same as that of the original grass. This result is in contrast to that for cotton (Table I), where the undigested residue showed a d.p. that steadily decreased with time in the rumen. In the experiments reported in Table IV, however, the d.p. measurements are on α-cellulose which has, of course, been extracted with 10% aqueous sodium hydroxide and all the fraction of low molecular weight thus removed. The d.p. results for α-cellulose (Table IV) and also the results for hemicellulose viscosity (Table II) are reminiscent of those reported for degradation of wood by a white-rot fungus, *Polyporus versicolor*[25]. Here also, there was only a slight decrease in the d.p. of holocellulose, which indicated that the organism degraded crystalline and amorphous cellulose simultaneously and at rates proportional to the amounts present. In contrast, attack by a brown-rot fungus was found[25] to result in a rapid fall in the d.p. of the holocellulose, and the difference has been postulated as due to possible penetration of the microfibrillar fine-structure by cellulolytic enzymes from the brown-rot, but not from the white-rot, fungus. In our work, however, we must assume that similar enzymes are involved in the digestion of both cotton and grass. The difference is probably associated with the presence of lignin in the grass, but the detailed mechanism of the effect is not obvious at present. Further investigation of this situation might be most effectively carried out by a detailed microscopic examination of the different fibres during digestion.

## EXPERIMENTAL

*Animals, feeds, and feeding conditions.* — Rumen-fistulated steers (Droughtmaster breed) were housed in a roofed pen and fed once daily with 5–6 kg (dry matter per animal) of a hay consisting of Townsville stylo and spear grass (1/1). The animals were preconditioned in this way for 3 days prior to the digestion experiments.

*Digestion experiments.* — The α-celluloses, hemicelluloses, holocelluloses, and faeces fibre were collected as described earlier[15]. Raw cotton (kindly supplied by Dr. C. M. Stewart, C.S.I.R.O., Melbourne) was purified by boiling for 8 h with 1% aqueous sodium hydroxide[26]. Samples (2 g) were enclosed in Terylene bags and kept

in the rumen as described earlier[27]. Five bags were removed at several time intervals between 0 and 136 h, washed well in running water (20 min), and freeze-dried. The *in vitro* digestibility of cotton was determined as described by Minson and McLeod[28], omitting the pepsin-digestion step. Samples were removed at several time intervals between 0 and 216 h, washed with water (4×), centrifuged, and dried *in vacuo* at 40°. Samples of faeces fibre (3 g), faeces holocellulose (3 g), and spear-grass holocellulose (1 g) were enclosed in Terylene bags and suspended in the rumen. Two bags of each material were removed at specified times, and washed and dried as above.

*Analytical procedures and methods.* — Viscosities were determined by using an Ubbelohde viscometer at 25 ±0.05°. A Rigaku Denki X-ray diffractometer was used with a stabilized bi-plane X-ray generator and horizontal wide-angle goniometer. The experimental curves were made using Ni-filtered $CuK_\alpha$ radiation, $\lambda = 1.5418$ Å with a tube voltage of 40 kV/20 mA, scanning speed 0.5°, $2\theta$/min with a time constant of 4. The chart speed used was 10 mm/min, and full-scale deflection on the chart recorder was set by the ratemeter at 128 or 250 c.p.s. The samples were smear-mounted onto glass slides, using acetone as the mounting medium. Prior to mounting, the samples were cut to a powder using surgical scissors. The samples were run over the angular range of 5.40°, $2\theta$.

Cadoxen [tris(ethylenediamine) cadmium dihydroxide] was prepared as follows: a 28% aqueous solution (w/w) of fresh ethylenediamine (redistilled) was cooled to 5°. Cadmium oxide (10% by weight based on ethylenediamine) was added to the cooled solution and stirred (24 h). Excess of cadmium oxide and cadmium hydroxide was removed by centrifugation. The cadmium concentration of the solution (measured by evaporating to dryness a known volume of solution and fusing the dried residue at 600° to cadmium oxide) was adjusted to 4.7–4.8% by the addition of aqueous (28%) ethylenediamine. The solution was stored at 5°. Cellulose samples (~25 mg) were wetted with 10% NaOH (0.3 ml, 20 min) at 5°, and cadoxen (25 ml) was added. The solutions were stirred (3 h) and centrifuged (2000 *g*, 10 min), and the supernatant solution was immediately added to the viscometer. Any undissolved material was washed twice with 0.02M HCl and twice with ethanol, and then dried to constant weight *in vacuo* (40°). This weight was subtracted from that of the starting material in order to calculate the final concentration. The d.p. was calculated from a single viscometry reading by substituting in the following equation[29]:

$5.6 \times 10^{-3}$ d.p. $= [\eta_{sp}/c]/[1 + 0.28\,\eta_{sp}]$, where $\eta_{sp} = (t - t_0)/t_0$, and c = concentration in g/100 ml.

Solutions of hemicelluloses [250 mg in M KOH (25 ml)] were stirred under nitrogen (10 min), centrifuged (2000 *g*, 10 min), and placed in the viscometer under nitrogen. Dilutions were made by the addition of M KOH. The small amount of undissolved material was washed twice with ethanol and dried *in vacuo* to constant weight (40°). This weight was subtracted from the starting weight in order that the final concentration could be calculated. The results were expressed as viscosity number ($\eta_{sp}/c$), with c in g/ml.

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