PHYSIOLOGIA PLANTARUM VOL. 23, 267–277, 1970

A. R. SHELDRAKE and G. F. J. MOIR

A Cellulase in *Hevea* Latex

Reprinted from
PHYSIOLOGIA PLANTARUM

Official publication of the Scandinavian Society for Plant Physiology
Editorial office: Department of Plant Physiology, S-223 62 Lund, Sverige

PHYSIOLOGIA PLANTARUM, VOL. 23. 1970, 267–277

A Cellulase in *Hevea* Latex

By

A. R. SHELDRAKE and G. F. J. MOIR

The Rubber Research Institute of Malaya
P.O. Box 150, Kuala Lumpur, Malaysia

(Received May 13, 1969)

Abstract

Using a viscometric method the latex of *Hevea brasiliensis* was found to contain a highly active cellulase capable of hydrolysing carboxymethyl cellulose. The enzyme has a pH optimum of around 6.3. It is present in the serum of the latex and is not membrane-bound to any significant extent. Similar cellulase activities were detected in latex from old and new latex vessel rings and also in latex from regularly tapped vessels and newly tapped vessels. The possible role of the enzyme in the removal of cell wall material during the differentiation of latex vessels is discussed.

Introduction

The first report of the existence of cellulase in higher plants, other than in germinating seeds and seedlings, appears to be that of Tracey (1950). Subsequent work has confirmed its presence in other higher plant tissues (*e.g.* Hall 1963, Maclachlan and Perrault 1964, Sassen 1965). However, work on the enzyme so far has involved a demonstration of its activity in extracts or homogenates of tissues containing a variety of cells, and it is not possible to decide from the available evidence in which cell types the enzyme may be produced and active. This point is of some interest in relation to the hypothesis of Fan and Maclachlan (1966) that cellulase is involved in the auxin-stimulated growth of cells by weakening primary walls. An additional, or alternative, explanation of the presence of cellulase in higher plant tissues is that it might be concerned with the process of removal of cell wall material during differentiation. Two instances of this process are the disappearance of end walls in differentiating xylem vessels and the disappearance of end walls during the differentiation of many types of articulated laticifers (Esau 1967). We have found (Sheldrake 1969) that latex from various plants

with articulated laticifers contains cellulase, while no cellulase was detected in latex from plants with nonarticulated laticifers which develop by intrusive growth without cell fusion or wall breakdown (Esau 1967). This evidence suggests that cellulase is involved in the removal of wall material during cell differentiation. *Hevea brasiliensis* has articulated laticifers and readily available latex with high cellulase activity, making it a favourable source of material for more detailed investigations of this enzyme.

Materials and Methods

Latex was obtained from trees of *Hevea brasiliensis* growing at the Rubber Research Institute's Experiment Station, at Sungei Buloh, near Kuala Lumpur. The trees had been tapped on the half-spiral alternate daily system (Riches and Gooding 1952) for several years. Unless otherwise stated, latex from clone RRIM 600 was used. It was collected for 30 minutes after tapping into vessels cooled by ice and centrifuged soon afterwards.

Carboxymethyl cellulose, sodium salt (CMC) was obtained from British Drug Houses, Ltd., and Triton X-100 from Sigma Chemical Co.

Visking tubing was used for dialysis.

Centrifugation was carried out in a Spinco Model L ultracentrifuge essentially as described by Moir (1959). This separates the latex into three major phases: a cream of rubber particles moving centripetally, an aqueous "serum" and a sediment known as "bottom fraction". Serum was run off by puncturing the cellulose nitrate tubes.

Cellulase was assayed viscometrically using CMC as substrate in a similar manner to that described by Tracey (1950). The flow time in Ostwald viscometers kept in a water bath was measured with a stopwatch. Unless otherwise stated, measurements were made at 22°C. The reaction mixture consisted of 4 ml 0.5 *M* phosphate buffer pH 6.0, 4 ml 2 % (w/v) CMC solution, latex serum, and water to give a final volume of 20 ml. To obtain readings within a suitable range, usually between 0.1 and 1.0 ml serum were used. Flow times were measured at times such that when half the flow time had elapsed, approximately 5 and 15 minutes had passed since the enzyme and substrate were mixed. The flow times at exactly 5 and 15 minutes were then obtained from a graph and were used to calculate cellulase activity in units similar to those of Tracey (1950), *i.e.*, one cellulase unit was defined as the enzyme activity which led to a decrease of viscosity of the reaction mixture such that the $\log_{10}$ of the viscosity increment at 5 minutes and the $\log_{10}$ of the viscosity increment at 15 minutes differed by 0.01. The total amount of acitivity in an incubation was kept in the range of 3–6 units by adjusting the volume of latex serum added. The activity of different samples was compared by calculating the number of units which would have been present if 5 ml of serum had been used in the standard reaction mixture.

Reducing sugars were estimated by the Nelson-Somogyi method (Hodge and Hofreiter 1962).

Results

Cellulase activity

The viscosity of the CMC reaction mixture declined so rapidly in initial trials that there was a viscosity decrease of over 40 % before the first reading of flow time could be taken. The amount of serum used had to be reduced considerably before activity could be conveniently measured. Figure 1 shows the change in viscosity of a mixture of serum and CMC. There was no measurable viscosity change due to the serum alone, and no change in viscosity occurred when the CMC mixture was incubated without serum; a very slight decrease took place in the presence of boiled serum (Figure 1).

An incubation was carried out at 25°C instead of the usual 22°C using conditions identical to those of Tracey (1950) in order to obtain a direct comparison of the cellulase activity in his samples and in latex serum. A sample of latex serum from clone RRIM 600 had an activity under these conditions of 235 of Tracey's units/ml.

The release of reducing sugars from CMC could be demonstrated using either dialysed or undialysed serum. Dialysis, however, led to a considerable reduction in activity (Table 2). Undialysed serum has a high blank reducing sugar reading; the blank reading of serum controls changes on incubation, presumably since both invertase and sucrose are present as are also the enzymes of glycolysis (Bealing 1969, Tupy and Resing 1969, d'Auzac and Jacob 1969). However, both in the presence and absence of NaF which blocks glycolysis in the concentration used here (Tupy and Resing 1969) a con-

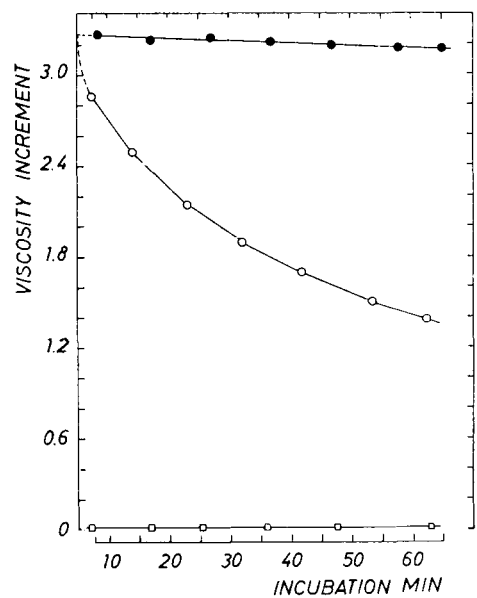

Figure 1. *Cellulase activity.* Serum (0.25 ml) from latex of clone RRIM 519 was incubated in the usual 20 ml reaction mixture with (○) or without (□) CMC. Serum (0.25 ml) which had been placed in a boiling water bath for 10 min was used as boiled enzyme control, with CMC (●).

Table 1. *Release of reducing sugars from carboxymethyl cellulose* (CMC) *by latex serum.* The complete reaction mixture contained 0.3 ml 2 % CMC, 0.2 ml 0.5 *M* phosphate buffer pH 5.8 and 0.5 ml serum. The increases in reducing sugars in each reaction mixture after incubation for 2½ h at 35°C are shown below in µg glucose equivalents. The figures given were obtained after subtracting the amount of reducing sugar in identical reaction mixtures kept at 0°C for the same period.

Reaction mixture	Without NaF	With NaF 5×10^{-2} *M*
CMC control ..... (mixture without serum)	0	0
Serum control ..... (mixture without CMC)	26	100
Serum + CMC ..... (complete mixture)	418	787
Difference	+392	+687

siderable increase in reducing sugars occurred on incubation with CMC. Table 1 shows the results of a typical experiment.

The high blank reading in incubation mixtures made the unequivocal demonstration of reducing sugar release from swollen and native cellulose impossible.

The pH optimum of the enzyme assayed viscometrically with CMC as substrate is around 6.3. The activity of the enzyme falls off more rapidly at pH values below the optimum than it does at values above the optimum (Figure 2).

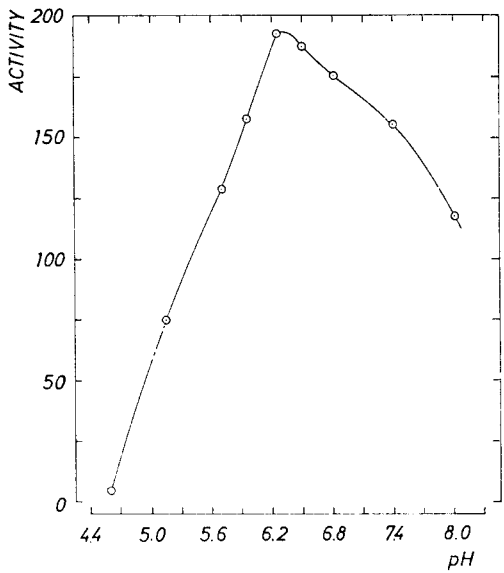

Figure 2. *pH optimum curve of cellulase at 22°C.* Serum from latex of clone RRIM 600 was used. Each reaction mixture (20 ml) contained 6.0 ml of McIlvaine citrate/phosphate buffer. The pH of each sample was checked, after incubation, with a pH meter. Activities are expressed as units per 5 ml of serum (see text).

Table 2. *Effect of various treatments on the cellulase activity of serum.* The activities are expressed as percentages of the activity of serum assayed shortly after centrifugation of the latex.

Control	100.0
Serum stored 24 h at 4°C	96.0
Serum stored 240 h at 4°C	69.5
Serum dialysed for 24 h at 4°C	49.5[1]
Serum incubated with dialysis tubing for 24 h at 4°C	87.5
Serum placed in boiling water bath for 10 min	< 1

[1] After correction for dilution during dialysis.

The activity of the enzyme did not decrease rapidly when the serum was stored at 4°C (Table 2).

The decrease of activity on dialysis may, to a small extent, be due to an inactivating or adsorbing effect of the cellulose dialysis tubing, since a decrease occurred simply on incubation with Visking tubing (Table 2). The larger part of the decrease during dialysis could be explained by the precipitation and flocculation of serum proteins that occurred during dialysis; but some loss of co-factors or activators may also have taken place.

Measurements of cellulase activity in the serum of latex from different clones were made on a number of occasions. The activities in units/5 ml serum are as follows, with the number of samples tested in brackets and the mean deviation.

Clone	RRIM 600	(6)	148 ± 10.0
Clone	RRIM 519	(4)	158 ± 28.0
Clone	Tjir 1	(8)	37 ± 4.5

The differences between clones RRIM 600 and 519 are not significant, but the values for Tjir 1 show that a large difference can exist between clones.

Effect of filtration

Filtering a small volume of serum through filter paper led to a decline in cellulase activity of over 99 %. Only a slight loss of activity occurred on filtration through sintered glass. This suggested that the cellulose of filter paper might be adsorbing the cellulase strongly, rather than that the enzyme was bound on particles too large to pass the filter paper. This was checked in two further ways. Firstly: small strips of filter paper were placed for 15 minutes in a sample of serum which was then assayed for cellulase activity. This declined to 57 % of the control, presumably due to adsorption of cellulase on cellulose. Secondly: serum was run out of a centrifuge tube in such a way that the lowest, middle and top thirds of it were collected separately. Taking the cellulase activity of the lowest fraction as 100, the activity of the middle fraction was 98 and of the top fraction 102, showing that the enzyme is homogeneously distributed in serum after centrifugation. If it were bound to particles, it would be expected to become unequally distributed in a centrifugal field.

The distribution of cellulase in whole latex

It has recently been shown by Pujarniscle (1968) that the lutoids (the major particulate component of the bottom fraction of centrifuged Hevea latex) contain a number of hydrolytic enzymes which can be released by osmotic shock or by the non-ionic detergent Triton at a concentration of 0.03 %. Experiments were conducted to find out whether cellulase is present in a membrane-bound form in the bottom fraction of latex.

Bottom fraction from 94 ml of centrifuged latex was resuspended in 85 ml of 0.025 *M* phosphate buffer pH 7, containing 0.4 *M* mannitol. 80 ml of this suspension were re-centrifuged in order to wash the particles. The supernatant was discarded and the bottom fraction was once more suspended in mannitol-phosphate buffer (60 ml). This was divided into 3 portions: one served as control, one was frozen and thawed twice, and to the third Triton X–100 was added to a final concentration of 0.1 %. The samples were then centrifuged and the supernatants were assayed for cellulase activity. 5 ml of each supernatant were used in the usual 20 ml reaction mixture. No activity was detected in the control, 0.7 units were found in the frozen and thawed sample, and 2.4 units in the Triton X–100 treated sample. The total amount of cellulase activity released by Triton X–100 from the bottom fraction of the original 94 ml latex sample can be calculated and is about 30 units. This compares with a total cellulase activity in the serum of the same sample of about 1000 units. Thus the enzyme activity in the bottom fraction released by Triton X–100 is about 3 % of the activity in the serum. This suggests that the enzyme is not specifically localised in the bottom fraction, but is slightly and unspecifically adsorbed.

The possibility remained that the enzyme was adsorbed or membrane-bound in some other fraction of the latex. To investigate this, Triton X–100 was added to latex at two concentrations 0.4 % and 0.8 %, before centrifugation. These relatively large amounts were used because it was found that the rubber particles had a high adsorptive capacity for the detergent and reduced its effective concentration in whole latex. Cellulase was assayed in the serum of latex that had been treated as described above. The results are shown in Table 3. It can be seen that the cellulase activity per unit volume of serum decreases after treatment with Triton X–100. This cannot be explained by an inhibitory effect of the detergent because in a separate experiment it was found that cellulase activity is unaffected by Triton X–100 at these concentrations. The decline in cellulase activity per unit volume can be accounted for by the dilution of latex serum with fluid released from the lutoids of the bottom fraction: the *total* amount of cellulase activity in the

Table 3. *The effect of Triton X-100 on whole latex.*

Parameter	Cellulase activity in 5 ml serum	Volume of serum from 94 ml latex ml	Total cellulase activity in serum
Control	164	30.6	1003
Triton X–100 0.4 %	156	34.5	1076
Triton X–100 0.8 %	129	42.5	1096

Triton treated samples is only slightly greater than that in the control; this slight increase could be due to release of unspecifically adsorbed enzyme from membranes of bottom and top fractions.

The results of these experiments show that nearly all the cellulase activity in latex is present in the serum.

Cellulase activity in latex from new and old vessel rings

Latex vessels in the bark of Hevea are laid down in the phloem region as a result of cambial activity. The younger rings of vessels are thus close to the cambium and the older ones are further out in the bark (Riches and Gooding 1952). In order to compare the cellulase activity of latex from new and old latex vessel rings, latex was obtained from matched groups of three trees each, one group being tapped close to the cambium and the other group tapped in the outer part of the bark, in each case on an established tapping cut. On a subsequent occasion the reverse was carried out: *i.e.* the trees which had previously been tapped close to the cambium were now tapped in the outer part of the bark, and vice versa. On centrifugation the latex from inner tappings usually showed more yellow colour in both top and bottom fractions, and especially in the bottom fraction. This is probably due to a higher content of Frey-Wyssling complexes. (This interpretation agrees with unpublished observations by Dr. W. A. Southorn of this Institute. For a description of Frey-Wyssling complexes see Dickenson, 1965 and 1969). The cellulase activities of the serum from the latex samples are shown in Table 4. These results do not establish any consistent difference between the cellulase activities of latex from inner and outer tappings. The activities are of the same order (apart, of course, from the clonal difference between Tjir 1 and RRIM 519).

Cellulase activity in latex from new and old tapping panels

Latex from a repeatedly tapped vessel system differs in a number of respects from latex from a new tapping cut (Riches and Gooding 1952). Latex was collected from the normal tapping panels of individual trees which had

Table 4. *Cellulase activity in latex from new and old vessel rings.* The activity in units/5 ml serum is given. In each experiment two groups of 3 trees each were used, and those which were tapped in the inner part of the bark on the first occasion were tapped in the outer part on the second occasion, and vice versa.

Date of tapping	Cellulase activity	
	Inner tapping	Outer tapping
Experiment 1 22/11/68	36	35
Clone Tjir 1 4/12/68	28	38
Experiment 2 6/12/68	34	49
Clone Tjir 1 12/12/68	43	38
Experiment 3 6/12/68	214	163
Clone RRIM 519 12/12/68	149	110

Table 5. *Cellulase activity from old and new tapping cuts.* Single trees from Clone Tjir 1 were used in each experiment. Activity is expressed as cellulase units per 5 ml serum.

Exp. no.	Cellulase activity		Ratio $\frac{\text{new}}{\text{old}}$
	Old tapping panel	New tapping panel	
1	35	28	$\frac{80}{100}$
2	43	34	$\frac{79}{100}$
3	44	35	$\frac{80}{100}$

been regularly tapped for several years and simultaneously latex was collected from a new tapping panel cut on virgin bark higher up on the other side of each tree. As expected, the yield of latex from the new tapping cuts was much lower, the latex coagulated faster and it was yellower in all cases. Cellulase was assayed in the serum of the latices thus obtained (Table 5). Although the cellulase activity in latices from new panels is somewhat lower in all cases the difference is not great. This result disposes of the possibility that the enzyme is produced by micro-organisms on the tapping cuts of the trees. No significant population of such organisms would be expected in a fresh cut on virgin bark.

Discussion

The cellulase activity of *Hevea brasiliensis* latex appears to be remarkably high. The activities found in extracts of pea epicotyls by MacLachlan and Perrault (1964) and in tomatoes by Hall (1963) were obviously very much lower. The activities measured by Tracey (1950) in the expressed sap of tobacco plants can be directly compared with the cellulase activity found in latex because an assay was carried out under the conditions specified by Tracey and the results were calculated in his units. He found 1.4–4.4 units/ml in stem and leaf sap and 12 units/ml in root sap. A sample of latex serum had a cellulase activity in Tracey's units of 235 units/ml. The relatively high activity of cellulase in Hevea latex would make this a good source of material for further studies on higher plant cellulase, about which little is known. In our experiments, in common with almost all others of which we are aware on cellulase in tissues of higher plants, an attack on unmodified native cellulose has not been demonstrated. Work on microbial cellulase has shown that activity assayed with CMC as a substrate (CM-cellulase activity) is only one component of a cellulase complex (Reese Siu and Levinson 1950; Gascoigne and Gascoigne 1960; Wood 1968); whether a similar complex exists in higher plants is not known.

The electron microscopic observations of Sassen (1965) on the differentiation of the articulated laticifers of *Achras sapota* show that the dissolution of wall material occurs extra-cellularly: the living cytoplasm of the laticifer is

separated from the wall by the plasma membrane, which is in close contact with the end walls during their dissolution. Electron micrographs of laticifer differentiation in Hevea show that a similar process takes place (Southorn and Gomez, personal communication). Sassen (1965) observed electron-dense bodies between the plasma membrane and the dissolving end walls of the cells after $KMnO_4$ fixation and suggested that these play a part in the removal of wall material. No observations on comparably fixed Hevea material are available. However it is possible to speculate that reverse pinocytosis of vesicles or particles is likely to be involved in the export of wall hydrolysing enzymes across the cell membrane, since molecules as large as enzymes are not thought to move out of cells by simple diffusion. It is therefore at first sight puzzling that the cellulase of Hevea latex is not present in a particle or membrane-bound form, but that the great majority of it is present as a soluble enzyme in the serum. One possible explanation is that it is present in a membrane-bound form at the time of differentiation but is released into the cytoplasm as the laticifer matures. Another possible explanation is that the enzyme detected here acts on a substrate that is not extracellular but intracellular. It has already been mentioned that CM-cellulase activity assayed here has been found in certain micro-organisms to be only part of a cellulase complex. Two of the other components, whose activity can be separated from CM-cellulase (Gascoigne and Gascoigne 1960) are concerned with the swelling of native cellulose ('S factor') and the degradation of insoluble highly-ordered cellulose ('C_1 enzymes'). The CM-cellulase acts synergistically with these by hydrolysing the short-chain cellulose molecules released by the activity of the other two factors. If a similar cellulase complex exists in higher plants, including Hevea, it can be imagined that C_1 and S factor activities might operate extracellularly, in the cell wall itself, releasing shorter partially hydrolysed cellulose molecules which could move across the plasma membrane and become the intracellular substrate for the CM-cellulase of latex. The digestive cellulase of snails, many fungal cellulases and cellulase of germinating seeds have relatively low pH optima (Gascoigne and Gascoigne 1960). The pH optimum of the latex enzyme is near neutrality, and the pH of the latex is also close to 7, so an intracellular CM-cellulase activity of the type suggested here is feasible. One prediction of this hypothesis is that C_1 and S factor activities, if present in latex, should be particle or membrane-bound.

The similarity of the activities of the CM-cellulase in latex from new and old tapping cuts and the similarity of the enzyme activity in the 'outer' and 'inner' tapped latices are basically the same phenomenon, since latex tapped from outer parts of the bark comes from old vessel rings, regularly tapped for some time, and at least some of the latex from the 'inner' tapping close to the cambium comes from previously untapped vessel rings. But if cellulase is concerned with cell differentiation, why is it not diluted out of latex from old vessel rings which have been tapped regularly, and present in much higher amounts from previously untapped and newly differentiated vessel rings? One possibility is that cell differentiation is necessary for latex production; if laticifer differentiation continues in old vessel rings, this could account for the presence of cellulase in latex from them, and the near equality of cellulase activity in latex from 'inner' and 'outer' tappings could be explained

on the basis that an equal number of cells have to differentiate for a unit volume of latex in each case. This resembles the postulation of Dickenson (1969) on the basis of ultrastructural studies, that new rubber particles and lutoids may only arise in differentiating cells, which would entail the continued differentiation of laticifers in continuity with any vessel system that continued to yield latex. However there is not enough anatomical information available to test these views. An alternative explanation for the continued production of cellulase in old latex vessel rings is that it is produced not only at the stage of cell differentiation but also in mature laticifers. Since these contain living cytoplasm and nuclei (Dickenson 1965) protein synthesis can proceed in them, and it may be that once the genetic material for cellulase production has been derepressed at the beginning of differentiation it is not repressed again once the dissolution of end walls is complete.

Another problem in laticifer differentiation concerns the mechanism by which the end walls are selectively removed; why are all the walls not dissolved to an equal extent? Numerous problems exist concerning the differentiation of other cell types in higher plants: but such problems may be more amenable to experimental attack with laticifers than with other cells: laticifers offer the unique advantage that ultrastructural studies can be correlated with biochemical investigations on their differentiated cytoplasm, available from Hevea, by tapping, in virtually unlimited amounts.

We thank Mr. J. F. McEwen and Mr. S. E. Chua for their co-operation; Dr. W. A. Southorn and Dr. J. B. Gomez for unpublished data; Mr. S. Sivanayagam, Mr. Y. H. Lee and Mr. M. Gopalsami for technical assistance. One of us (A.R.S.) wishes to thank Mr. B. C. Sekhar, the Director of the Rubber Research Institute, for hospitality and gratefully acknowledges a Royal Society Leverhulme Scholarship.

Present address of A. R. Sheldrake: Department of Biochemistry, University of Cambridge, England.

References

d'Auzac, J. & Jacob, J-L.: Sur la régulation de la glycolyse dans le latex d'Hevea brasiliensis. — J. Rubb. Res. Inst. Malaya 21 Part 4. 1969.

Bealing, F. J.: Carbohydrate metabolism in Hevea latex: availability and utilization of carbohydrate substrates. — *Ibid.* 21 Part 4. 1969.

Dickenson, P. B.: The ultrastructure of the latex vessel of Hevea brasiliensis. — Proc Nat. Rubber Producers, Research Association Jubilee Conf. Cambridge 1964, pp. 52–66. Maclaren and Sons Ltd, London. 1965.

— Electron microscopical studies of the latex vessel system of Hevea brasiliensis. — J. Rubb. Res. Inst. Malaya 21 Part 4. 1969.

Esau, K.: Plant Anatomy. — 2nd ed. John Wiley and Sons, New York. 1967.

Fan, D. F. & Maclachlan, G. A.: Control of cellulase activity by indoleacetic acid. — Can. J. Bot. 44: 1025–1034. 1966.

Gascoigne, J. A. & Gascoigne, M. M.: Biological Degradation of Cellulose. — Butterworths, London. 1960.

Hall, C. B.: Cellulase in tomato fruits. — Nature (Lond.) 200: 1010–1011. 1963.

Hodge, J. E. & Hofreiter, B. T.: Determination of reducing sugars and carbohydrates. — *In* Methods in Carbohydrate Chemistry, Vol. I (Whistler, R. L. and Wolfrom, M. L., eds.). Academic Press, New York. 1962.

Maclachlan, G. A. & Perrault, J.: Cellulase from pea epicotyls. — Nature (Lond.) 204: 81–82. 1964.

Moir, G. F. J.: Ultracentrifugation and staining of Hevea latex. — *Ibid.* 184: 1626–1628. 1959.
Pujarniscle, S.: Caractère lysosomal des lutoides du latex d'Hevea brasiliensis Mul. Arg. — Physiol. Vég. 6: 27–46. 1968.
Reese E. T., Siu, R. G. H. & Levinson, H. S.: The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. — J. Bact. 59: 485–497. 1950.
Riches, J. P. & Gooding, E. G. B.: Studies in the physiology of latex. I. Latex flow on tapping — theoretical considerations. — New Phytol. 51: 1–10. 1952.
Sassen, M. M. A.: Breakdown of the plant cell wall during the cell-fusion process. — Acta Bot. Neer. 14: 165–196. 1965.
Sheldrake, A. R.: Cellulase in latex and its possible significance in cell differentiation. — Planta (Berl.) 89: 82–84. 1969.
Tracey, M. V.: Cellulase from leaves and roots of tobacco. — Biochem. J. 47: 431–433. 1950.
Tupy, J. & Resing, W. L.: Substrate and metabolism of carbon dioxide formation in Hevea latex in vitro. — J. Rubb. Res. Inst. Malaya 21 Part 4. 1969.
Wood, T. M.: Cellulolytic enzyme system of Trichoderma koningii. Separation of components attacking native cotton. — Biochem. J. 109: 217–227. 1968.