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Physiology of Hevea (Latex Flow)

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Hevea brasiliensis *latex is a cytoplasm contained in vessels with elastic and permeable walls. The latex is at high pressures in the vessels before tapping. Latex flows because of contraction of the vessels and movement of fluids into the vessel after tapping. Latex flow is impeded by 'plugging' which occurs close to the vessel ends. Electron microscopy shows plugs of coagulated rubber within the vessels in which there are damaged lutoids which will have spilled their contents. The contents of the lutoid, if released, could initiate plugging. The clotting mechanism and factors which could provoke release of the clotting system are discussed.*

Growth regulators such as 2,4,5-T prevent or delay plugging in ways not yet understood. Other chemical treatments which affect flow are listed. It is suggested that the tree can limit its losses of fluid by two distinct mechanisms, firstly by lutoid-induced plugging, provoked by shear and other agencies during fast flow, and secondly, by general loss of turgor followed by coagulation on the cut when flow rate is slow. The first mechanism is most effective for small wounds, the second is a reserve mechanism which stops flow from large wounds. Prospects for applications of recent work are discussed.

When *Hevea brasiliensis* is wounded by tapping, latex flows for a time and then stops. Much work has been done over the years on the factors promoting and hindering flow. Early studies by BOBILIOFF (1923), ARISZ (1928), FREY-WYSSLING (1932), RICHES AND GOODING (1952) and SCHWEIZER (1953) established many important concepts. Recently the physiology of latex flow has again become a very active field of research, and it is timely to attempt a review of progress, particularly of the newer work.

Latex is contained in an articulated system of tubular vessels in the phloem tissues of *Hevea*. New vessels are generated at intervals from the cambium, at first as individual specialised cells which later join together to form networks of tubes by anastomosis. Many of the original cell nuclei are lost in the process, nevertheless the functioning latex vessel still retains many nuclei rather variably spaced along the length of the vessel. Traces of the lost cell walls can also be seen as thickenings of the vessel wall. Thus the latex vessel is not a smooth bored uniform tube, it has constrictions and obstructions in it which may influence flow. Since the latex vessels are laid down at intervals, they form sheaths or rings external to the cambium. Though there are numerous inter-connections between the vessels of one ring, there are virtually no connections between one ring and its neighbours. As the tree grows the vessels of the outer rings are broken up and lost among the sclerotic tissues of the outer bark. The vessels of rings near to the cambium are younger and of smaller diameter than those further out. The internal diameter of the latex vessel thus varies within the same tree; there are also differences between trees (GOMEZ *et al.*, 1968). However, as a rough approximation, one may take 30 μ as a not untypical value for the internal diameter of a productive vessel.

When a tree is first brought into tapping, a relatively small volume of viscous, concentrated latex is obtained. Successive tappings thereafter lead to a much longer flow of more dilute latex with increased yield of rubber. For an individual tapping the latex flow is at first rapid, then diminishes to a period of sluggish flow before stopping.

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Before tapping, latex is contained in the vessels at quite high pressures. ARISZ (1928) calculated turgor pressures of upto 10 atm from cryoscopic measurements. Recently BUTTERY AND BOATMAN (1964, 1966 and 1967) used direct techniques in which micromanometers of the type developed by BOURDEAU AND SCHOPMEYER (1958) are inserted into the latex vessels. They recorded values of 10–14 atm for turgor pressure in the vessels.

Flow behaviour is not fully explained by the hydrostatic pressures measured before tapping. One has to take into account the properties of cell walls, which are both elastic and permeable. ARISZ (1918) and FREY-WYSSLING (1929a) elaborated the concept that tapping causes a sharp fall in pressure within the vessel while neighbouring cells are still turgid. The latex vessel wall constricts, forcing latex out. At the same time water begins to flow across cell walls into the latex vessel to restore equilibria upset by the tapping. FREY-WYSSLING (1929a) stresses that these water exchanges will not be confined to cells adjacent to the latex vessel. They will spread throughout the phloem tissue and possibly into the xylem. Water passing into the latex vessel will dilute the latex, the process often being referred to as the 'dilution reaction' (ARISZ, 1920).

FREY-WYSSLING (1932 and 1952) attempted a mathematical treatment of latex flow considering the vessel system as a reservoir of latex formed of capillaries with elastic permeable walls. The elastic discharge ('extrusion flow') would be very rapid at first but the flow rate would fall off with time because, to reach the tapping cut, the latex would have to travel increasingly long distances through narrow-bore capillaries. The dilution reaction would alter the viscosity of the latex. In general terms Frey-Wyssling was able to offer explanations for most of the features of the flow curve observed in practice. He attributed the final cessation of flow to coagulation on the cut. RICHES AND GOODING (1952) criticised details of Frey-Wyssling's analysis and gave a different mathematical treatment; but they accepted the general concept.

Frey-Wyssling's concept of flow was widely accepted but speculation as to other possible mechanisms continued. The relatively large size of latex non-rubber particles attracted attention and various suggestions were made that they might agglomerate and plug latex vessels. TAYSUM (1957) proposed plugging by agglomeration brought about by bacteria of rubber, Frey-Wyssling particles (8 μ) and lutoids (2 μ). SOUTHORN (1961) considered osmotically swollen lutoids. TAYSUM (1961) suggested the formation of a constricting annulus of bacteria and destabilised latex particles at the top of a severed latex vessel.

The whole aspect of flow research was changed by observations made by BOATMAN (1966) and RUBBER RESEARCH INSTITUTE OF MALAYA (1963) that when a tree is tapped at frequent intervals on the same day (by removing approximately one millimetre of bark at each tapping) a 'stepped' flow curve is obtained. There is a marked recovery of flow rate after each tapping, indicating that an impediment to flow arises within the few minutes' interval between tappings, and that the impediment is located very near the vessel ends, since it is eliminated by removal of one millimetre of bark. BOATMAN (1966) and RUBBER RESEARCH INSTITUTE OF MALAYA (1963) also discovered that application of the yield stimulant 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) eliminated this obstruction to flow for the half-spiral tapping of a Tjir 1 tree. Repeated tapping of the stimulated tree no longer gave a stepped flow curve.

These important observations have led to many fruitful studies. Broadly speaking most of the new work has been along two lines: studies on the intensity of flow obstruction ('plugging') under different conditions, and fundamental studies on the nature of plugging.

PLUGGING INDEX

Boatman's repeated tapping curves suggested immediately the possibility of assigning an index to plugging intensity from the flow pattern. Many plugging indices have been considered with varying degrees of sophistication. The simplest, and the only one as yet widely studied, is that of PAARDEKOOPER AND

SAMOSORN (1969) and MILFORD *et al.* (1969). The most striking results obtained from this approach are that for half-spiral tapping in a given geographical area, plugging index is shown to be a clonal characteristic, and that yield stimulation is most effective with trees which show a high plugging index, as would be expected from Boatman's observations, that a major part of the yield stimulatory action of 2,4,5-T is to diminish plugging.

VESSEL CONTRACTION

Studies on the nature of plugging have followed several lines. First we will consider the effect of contraction of the vessel walls postulated by ARISZ (1920) and FREY-WYSSLING (1932). This would be a factor promoting flow by expulsion of latex, but there have been suggestions that the vessels might at some stage narrow in diameter to such an extent as to impede flow. Shrinkage of latex vessel diameters results in a reduction in the overall thickness of the bark. PYKE (1941) measured the reduction in diameter of the tree after tapping using a dendrometer. He found no contraction above the tapping cut, but a definite reduction in diameter one inch below the cut after tapping, attributable to collapse of latex vessels. GOODING (1952a and b) made improvements to Pyke's dendrometer and confirmed these observations. BOATMAN (1966) using a still further improved dendrometer concluded that the reduction in tree diameter could be accounted for if each vessel decreased in diameter by 3.5 to 5.7 μ. The mean bore of the vessels was over 20 μ so that such constriction would not close them. After Boatman's repeated tapping experiments, it became important to check whether a higher degree of local collapse of the vessels occurred very near the cut. FREY-WYSSLING (1932 and 1952) had suggested that after tapping the latex vessel wall profile assumed the shape of a truncated parabola, narrow at the vessel end. SOUTHORN (1967) devised new instruments to investigate changes in the thickness of the millimetre of bark just below the tapping cut after tapping. Contractions at the vessel ends were temporarily in excess of those occurring one inch below the cut, the difference amounting to 0.3 to 0.7 μ per vessel ring just after tapping and disappearing within ten minutes of tapping. These differences do not seem sufficient to account for the pattern of flow on repeated tapping.

LATEX STRUCTURE

Since localised vessel collapse does not explain plugging, possible plugging mechanisms in the latex were considered. The nature of latex is important in this context. For many years there was controversy as to whether latex was a cell-sap held in an enormously elongated vacuole (SCHMIDT, 1882; KALLEN, 1882; MOLISCH, 1901; FREY-WYSSLING, 1952) or a cytoplasm filling the vessel lumen (BERTHOLD, 1886; MILANEZ, 1946). The question was not resolved finally until it became possible to use electron microscopy to study latex vessel sections (ANDREWS AND DICKENSON, 1961; DICKENSON, 1965), when it became clear that latex is indeed a cytoplasm. In a vessel subjected to tapping, whereas most of the vessel lumen is filled with more dilute latex cytoplasm, there is a thin parietal layer of rather dense latex cytoplasm which remains in the vessel after tapping. The dense cytoplasm near the walls of the vessel would very slightly reduce the effective bore with respect to flow.

The latex which comes out contains a wide range of particles including rubber (SOUTHORN, 1961; COCKBAIN AND SOUTHORN, 1962) suspended in a serum. The serum was first separated by COOK AND SEKHAR (1953) using high-speed centrifugation; it became known as 'centrifuge serum' or C-serum.

By freezing and thawing the heavy 'bottom' centrifuge fraction, HSIA (1958) obtained a second serum, which is known as 'bottom fraction' serum or B-serum. MOIR (1959), using improved centrifuge techniques combined with staining, detected eleven centrifuge zones visible to the naked eye. Three types of particle together make up about 98% of the particulate volume. If any latex particle interferes with flow, it is likely to be one or more of these three. Rubber hydrocarbon particles are most abundant (25–45% by volume in latex) followed by lutoids (10–20%) and Frey-Wyssling particles (1–3%). The Frey-Wyssling particles (FREY-WYSSLING, 1929b) are yellow coloured

bodies, which under the electron microscope are seen to have a very complex structure (ARCHER *et al.*, 1963; DICKENSON, 1965 and 1969; SOUTHORN, 1966). The lutoids, first reported by HOMANS AND VAN GILS (1948) are heavy membrane-bounded bodies which, in centrifuged latex, are found mainly in Moir's Zone 8. Hsia's B-serum is the fluid serum contained within the lutoids, slightly contaminated by other material as originally prepared (SOUTHORN AND EDWIN, 1968). Latex is always contaminated by bacteria unless collected with elaborate precautions (TAYSUM, 1957, 1959 and 1961).

MICROSCOPY OF TAPPING CUT

SOUTHORN (1968a) examined the longitudinal sections of latex vessels near the tapping cut by optical and electron microscopy. Two possible sources of obstruction to flow were apparent. Firstly there was the cap of rubber coagulum which formed over the vessel ends and which often invades the vessel in the last stages of flow. Secondly, the vessels often showed internal plugs of coagulum, sometimes forming at different locations in the vessel near the cut. Bacteria, lutoids or Frey-Wyssling particles were not seen in sufficient numbers to form plugs by themselves and there was no trace of an annular constriction near the vessel ends as proposed by Taysum. Attempts were made to section vessels before flow had stopped. In some of these there is clear evidence of particle distortion along flow stream lines. Internal plugs seen in such sections either filled the vessel cross-section or were absent, suggesting that internal plugging, when it happens, is a swift process for a particular latex vessel—the plugs do not form slowly at the vessel wall and grow inwards. Though the plugging material was always coagulated rubber hydrocarbon, damaged lutoids were clearly visible in both the coagulum cap and the internal plugs. These lutoids would have spilled their contents (B-serum).

DESTABILISATION BY LUTOIDS

There have long been suspicions that lutoids were implicated in latex flocculation (PATON, 1953). Fresh latex collected in the field always contains microflocs in which rubber particles and lutoids are associated (SOUTHORN, 1961). If untreated, such latex coagulates under tropical conditions in 6 to 9 hours, mainly due to bacterial action (TAYSUM, 1957). However, even sterile latex loses its stability, given a longer time. MCMULLEN (1951) showed that latex collected aseptically deposited a heavy fraction (mainly lutoids) which flocculated spontaneously in a few days, whereas the remainder of the latex remained stable for weeks. All these reactions would appear to be rather mild and slow. SOUTHORN AND EDWIN (1968) showed that B-serum extracted from lutoids has an extremely fast and complete flocculating action on aqueous suspensions of rubber particles. In whole latex the situation is complicated by the fact that B-serum and C-serum react with each other. The effect of B-serum released into whole latex therefore depends on a balance of activities between the two sera. If the B-serum concentration is high enough in relation to the C-serum, then latex particles flocculate. In whole latex there is usually a sufficiently large amount of C-serum to prevent complete destabilisation of the latex even if all the lutoids were to leak, but whenever a lutoid disintegrates and discharges its contents, there would be temporarily a local high concentration of B-serum which produces a microfloc of destabilised particles. Microflocs can be produced in large quantities in whole latex by physical treatments such as ultrasonic irradiation which break the lutoids suddenly (SOUTHORN AND EDWIN, 1968). If lutoids are previously removed such treatments produce no microflocs.

These studies showed that latex contains its own 'built-in' clotting system capable of stopping flow if activated by the rupture of lutoids at the right time and place. The action of B-serum was at first thought to be entirely due to enzymes. The known proteins of B-serum include phosphatases and other hydrolytic enzymes (MCDONNELL, 1964; EDWIN, 1965; AUDLEY, 1965; PUJARNISCLE, 1965, 1966; PUJARNISCLE AND RIBAILLIER, 1966; ARCHER, 1962; ARCHER *et al.*, 1969). There are grounds for regarding the lutoid as homologous to the

lysosome of animal cells (PUJARNISCLE, 1968) and it has often been suggested that B-serum might lyse the colloidally protective envelope of the rubber particle, so destroying colloidal stability. Though enzyme activity of this sort may well exist in latex, SOUTHORN AND YIP (1968a) showed that the initial very fast clotting action of B-serum can be explained otherwise. B-serum has also a large content of cationically active material, both as divalent inorganic cations such as calcium and magnesium, and as proteins of high isoelectric point. C-serum on the other hand has a preponderance of proteins of relatively low isoelectric point and a low concentration of divalent metal cations. The difference in charge distribution of the proteins in the two sera show clearly in starch gel electrophoresis patterns (MOIR AND TATA, 1960; TATA AND MOIR, 1964; KARUNAKARAN *et al.*, 1961). The particles of latex are coated with colloidally protective envelopes, probably phospholipid protein in composition (COCKBAIN AND PHILPOTT, 1963). The particles are all negatively charged; this means that they mutually repel each other.

Release of B-serum into latex reduces this electrostatic stability factor; indeed, if enough B-serum is added, the charge at the shear interface of the rubber particle (ζ potential) can be brought from its normal -45 millivolts to near zero. B-serum and C-serum proteins also interact rather similarly and are thrown out of solution together, hence the moderating action of C-serum. Cationic surfactants in appropriate concentration have effects on latex stability similar to those produced by B-serum. It is possible to estimate the electrostatic activity of the B-serum quantitatively by its titration against anionic surfactants, or of C-serum by titration against cationic surfactants.

If free metallic cations are removed from B-serum by dialysis, the serum still retains most of its fast clotting activity, so that the proteins of B-serum play a major role. TATA AND YIP (1968) have shown that the protein activity pertains to a particular protein band of the B-serum starch gel electrophoresis pattern. They were able to separate a sufficient quantity of this material to demonstrate that it retained the clotting action of B-serum, and also that dialysed B-serum from which it had been removed showed no significant activity.

Let us summarise these recent advances concerning lutoids. We know that latex contains an integrated clotting system, normally segregated within the lutoid but active if released. We know a great deal about how this clotting system works and have methods to estimate serum-clotting activity quantitatively. We know that latex vessels after tapping are obstructed by coagulum which could well be formed by such a clotting system.

Micrographs of latex vessels after tapping show a thick coagulum cap over the severed ends of the latex vessels and internal coagulum plugs within the vessels, independent of the cap. There has been some debate as to the relative importance of cap and plug. Those who favour the cap as a dominant factor point out that its existence is evident to the naked eye whereas internal plugging is inferred from electron micrographs only. Apart from this, the cap obviously occurs very near the vessel ends as required to explain Boatman's repeated tapping experiments, whereas if internal plugs are important it is necessary to establish the existence of mechanisms inducing substantial leakage of B-serum within the flowing latex in the vessels and near to the severed ends.

The evidence against a dominant role for the cap in obstructing flow during the first few minutes of tapping seems rather strong. In the first place trees which have a very low plugging index, or which have been yield-stimulated so as to remove plugging, nevertheless form coagulum caps along the cut. Many experiments have been carried out in which cap formation has been prevented by washing the cut, with no detectable effects on flow (SOUTHORN, 1964). There would probably be general agreement that the coagulum cap is of importance in the last stages of very slow flow. If obstruction occurred only when flow rate became slow, then the cap would need to be considered seriously as the major impediment to flow.

On this subject, MILFORD *et al.* (1969) express the opinion that flow at low rates

under a low pressure gradient is the phase most affected by plugging, which would favour the cap hypothesis. This conclusion is by no means self-evident from their experimental results on repeated tapping of a Tjir 1 unstimulated tree. Marked plugging is apparent fifteen minutes from tapping, when flow rates are still quite high. BOATMAN (1966) takes the opposite view and concludes that plugging occurs during rapid flow. He found that stimulation, which removed or delayed plugging, also delayed fall off in flow rate. He makes an estimate of the time of onset of plugging from a comparison of flow curves between stimulated and unstimulated trees reopened at very frequent intervals, and concludes that flow becomes impeded within 1.5 minutes after tapping. SOUTHORN (1968a) points out that if internal plugging occurs, flow would probably cease abruptly in the vessel affected. Flow through the remaining exits would be faster per vessel than the total flow rate would indicate, this being the output of a diminishing number of open vessel ends. Obstruction would take place during the period of rapid flow for an individual vessel.

It is concluded that the plugging which usually occurs during the period of high pressure gradients cannot be ascribed to the coagulum cap, though the cap may play a part when flow has slowed down from other causes.

LUTOID STRUCTURE

There have been many studies of factors which might cause damage to lutoids. To understand these, it is first necessary to consider the lutoid in detail. It should be mentioned that lutoids in very young latex vessels differ in some respects from those in the older vessels which are subject to regular tapping and that the following discussion concerns work on the lutoids collected after tapping from mature vessels. The word 'collected' is important for though lutoids can be seen in sections of vessels, very few experiments on lutoids can be done within the vessels.

The lutoids are bodies bounded by extremely thin but complex membranes. Under the light microscope they are visible only when dark field or, better still, phase-contrast optics are used. They are then most often seen as spherical bodies in the range 2–10 μ diameter, often with particulate inclusions in lively motion (SCHOON AND PHOA, 1956; SOUTHORN, 1960). Thus the lutoid membrane encloses a colloid dispersion of particles in B-serum. It has already been shown that B-serum would promptly precipitate negatively charged particles. Hence the interior particles of the lutoid must have different surface properties and charge to the general run of latex particles. In fact fresh latex must be regarded as a dual colloid system. There is firstly a wide range of particles including rubber particles and lutoids dispersed in C-serum. All these particles normally carry a negative charge in their surface (SOUTHORN AND YIP, 1968a); they all move in the same direction towards the positive pole in a catephoresis cell. Within the interior of the lutoids there is a second colloid system in which particles must carry a more positive charge in order to retain their colloid stability. The two systems are colloidally antagonistic: if B-serum leaks into the C-serum dispersion it will promote clotting there. Similarly C-serum leaking through the lutoid membrane inwards would precipitate the B-serum dispersion. These two opposed systems are separated by the lutoid membrane which must have rather special properties.

We know that the exterior surface of a lutoid dispersed in C-serum carries a negative charge. The interior surface cannot be similarly charged in the presence of the cationic B-serum which it faces; it must carry approximately the same charge as the dispersed interior particles of the lutoid, otherwise these particles would come out of suspension by sticking to the wall at each collision. We thus have an electrically polarised membrane, the interior surface being positively charged with respect to the exterior. Polarised membranes are of course by no means uncommon in living cells. In a few cases, for very large cells such as are found in the animal nerve axon (HODGKIN, 1964) or the primitive plant *Nitella* (NAGAI AND KISHIMOTO, 1964), it is possible to investigate the polarisation in detail by inserting microelectrodes. So far this has proved impossible with the lutoid,

but a few simple experiments can be done using 'model' lutoids in which B-serum is placed in a cellophane bag immersed in C-serum (SOUTHORN AND YIP, 1965). If identical calomel electrodes are coupled through salt bridges across the cellophane membrane a small but finite difference can be picked up, the B-serum side of the membrane being positive to the exterior C-serum side. The cellophane membrane is a poor substitute for a lutoid membrane and the potential difference soon disappears. If platinum electrodes are placed across the cellophane membrane there is a very large potential difference, usually 200 to 300 millivolts. This is a redox potential, of considerable interest in view of Hsia's findings on the oxidase activities of B-serum (HSIA, 1958). This redox potential across a cellophane membrane also disappears after a short time. Concerning the origins of these potentials, the permeability of the membrane is important. The lutoid membrane separates two sera between which there are considerable disparities in ionic concentrations. For example, B-serum is acid with a pH of about 5.4 while C-serum is neutral, so that there is a large difference in hydrogen ion concentration. Calcium and magnesium are also ions concentrated in B-serum. Cellophane membranes do not maintain these differences for long. For example the pH difference between the two sera disappears within about a quarter of an hour in the cellophane bag experiments. The lutoid membrane can presumably maintain such concentration gradients provided it is not damaged. Donnan equilibria might account for some of the observations including some degree of electrical polarisation. However there are differences between the sodium and potassium concentrations of the two sera, and it seems very likely that the lutoid membrane has a variable permeability and is engaged in active transport. In many cases where it has been possible to insert microelectrodes to read accurate electrical potentials across living membranes, the phenomenon of 'action potential' has been observed. This arises from a polarisation affected by stimulation. It is a temporary change in electrical potential between 'stimulated' and 'resting' portions of the membrane. A membrane which produces such an effect is said to be 'excitable'. This appears to be a fundamental property of a great many living membranes developed to an extraordinary degree of specialisation in the animal nerve cell. The excitation stimulus can be osmotic, mechanical, electrical, chemical or thermal.

There is at the moment no solid experimental proof of excitability in the lutoid membrane but it is a possibility worth allowing for in considering factors which may affect lutoid membrane permeability. In the excited state after stimulation an excitable membrane is more permeable. For the lutoid this would mean a transfer of ions across the wall, and a decrease in the charge on the exterior surface with a corresponding decrease in electrostatic repulsion between individual lutoids. Including the possibility of 'excitability' lutoids are likely to react to adverse changes in external conditions in the following stages:

(a) Resting state: fully polarised with little tendency to agglomerate.

(b) Excited state: partly polarised with increased permeability and a slight tendency to mutual adhesion.

(c) Damaged state: inert depolarised membranes with impaired ultrastructure and greatly increased permeability. In hypotonic solutions, such lutoids would have a great tendency towards aggregation since small cations would tend to migrate to the outer surface. In hypertonic solutions this process would be reversed and the outer membranes might be less sticky.

(d) Grossly damaged state, in which the membrane disintegrates or develops large holes permitting free interchange of sera.

Conditions (a) and (b) would be reversible in the latex vessel and the lutoid would pass easily from one to the other according to the permeability conditions required by the cell. It is probable that after tapping the collected lutoids would mostly be in states (b), (c) or (d).

Let us see how this agrees with what is observed. When individual lutoids from latex

collected are watched under the phase-contrast microscope they 'die' and disintegrate after a time, sometimes quite abruptly, but often slowly in a characteristic way (SOUTHORN, 1960). First the interior particles disappear from suspension, they stick to the inner walls. This would correspond to complete loss of membrane polarisation [state (c)] so that the inner particles are no longer electrostatically repelled by the wall. Simultaneously the membrane thickens and becomes more easily visible. Finally the lutoid disintegrates [state (c) to (d)]. Sometimes before bursting, mass movement of material across a lutoid membrane can be detected by phase contrast microscopy as changes in refractive index of the lutoid serum.

PAKIANATHAN *et al.* (1966) observed that damage to latex 'bottom fraction' particles (mainly lutoids) is much greater in the latex which first emerges after tapping than it is in latex collected during the later stages of flow. This fits in well with the idea that whatever are the factors inducing lutoid damage they operate with greatest intensity during early flow when plugging is known to occur. It also suggests that if we wish to find lutoids in an undamaged state, as near as possible to how they might be in the latex vessel, then the best place to look would be in latex collected during the later stages of flow. A new way of observing some of the properties of the lutoid membrane has recently been introduced (SOUTHORN, 1968b). Lutoids in collected latex always show at least some tendency to agglomerate and form structures. These structures can be broken down in a suitable microviscometer, after which they form again. The rate of structure building can be followed by the changes in apparent viscosity shown. Since structure building depends on adhesion between the outer surfaces of the lutoid membrane, which will become more sticky if the membrane permeability increases, some information can be obtained about the membrane condition by this sort of experiment. Latex collected from the later stages of flow shows an interesting effect (YIP AND SOUTHORN, 1967). The rate of structure building attributable to lutoids increases after a time to a peak and then falls again. This must be due to a change in surface properties leading initially to a temporary increase in adhesion. The most likely explanation is that in the stage of increasing adhesion we are observing a transition from states (a) or (b) to (c) with increased permeability of the lutoid membrane. The subsequent decrease in adhesion may be due to the transition (c) to (d) or to changes brought about by the gradual establishment of new surface conditions following the change in permeability. The whole sequence of events can be followed only in latex from the later stages of flow. The early flow fractions show either a steady or a slightly decreasing rate of structure building—the last stages of the effect. The middle flow fractions display more of the process, often giving a very high rate of structure building followed by a decrease to a much lower value—the middle and later stages of the effect. The experiment indicates that intact lutoids undergo changes in surface properties which are far advanced in the latex first collected but have not progressed so far in the latex collected later.

When examined in section by electron microscopy the lutoid membrane is seen to be a 'unit membrane' (DICKENSON, 1965). The molecular structure of unit membranes is an important and much debated question. GOMEZ AND SOUTHORN (1969) have reported features of the lutoid membrane which would accord particularly well with the unit membrane model proposed by LUCY (1964). The lutoid membrane ultrastructure is modified and develops pores after treatment with chemicals such as saponin or neutral red. Behaviour of this sort has been reported for other biological membranes, for example, the membrane of the erythrocyte responds rather similarly.

FACTORS LIKELY TO DAMAGE LUTOIDS

The lutoid studies discussed now lead us to consideration of the factors which might damage lutoids during flow. The lutoid may exist in a number of conditions which we have provisionally labelled (a) through (c) culminating in (d) which is the onset of disintegration. The factors which could promote changes in the general direction (a) to (d) are thermal, electrical, osmotic, mechanical and chemical. We

shall not consider thermal factors since they are unlikely to be very important for practical purposes in this connection, though differences in temperature do have a small effect on lutoid membrane permeability (GOMEZ, 1968).

Electrical Effects

Whether electrical factors are worth considering depends firstly on whether they exist. LIM *et al.* (1969) showed that they do. Tapping *Hevea brasiliensis* results in an electrical wound potential which can be detected readily close to the cut. The possibility that wounding might affect flow by altering the permeability of excitable membranes, is a subject still unexplored but now worthy of investigation. It is intriguing that 2,4,5-T (a well known yield stimulant) appeared to have little effect on wound potentials, and that chloroform, which temporarily suspended wound response, has been reported to have a yield stimulating effect (BANCHI, 1967), while scraping the bark of a tree is known to stimulate yield. So far as effects on lutoids are concerned, wound potentials might well bring about depolarisation of the membrane [state (a) to (b)] as a local effect near to the vessel ends, which could sensitise the lutoids coming into this zone to further damage by agencies such as shear. This is a very attractive but as yet unproven hypothesis.

Osmotic Effects

The osmotic sensitivity of lutoids attracted attention soon after their discovery. If enough water is added to latex the lutoids burst. Most laboratory investigations on lutoids have been done at an osmotic concentration of between 0.35 and 0.4 M mannitol solution or equivalent as optimal for best preservation of lutoids separated from fresh latex (MOIR, 1957). Appreciably lower tonicities cause lutoids to swell and eventually to burst; higher tonicities do not usually break the lutoids but cause them to shrink (PAKIANATHAN *et al.*, 1966). The situation is complicated by the sensitivity of the lutoid membrane to the chemical composition of its environment, so that the choice of materials for adjusting pH or osmolarity is important (PAKIANATHAN *et al.*, 1966; GOMEZ, 1968). Mannitol solutions have been widely used for osmotic experiments because mannitol appears to be metabolically inert in latex, and because it has a relatively small effect on centrifugation behaviour (MOIR, 1957). Citrate buffers seem to be relatively innocuous to lutoids though phosphate, tris or veronal buffers have also been widely used. Materials which strongly adsorb on the lutoid surface such as cationic stains often accelerate lutoid breakage. Anionic surfactants (SOUTHORN, 1961) and water miscible lipid solvents are swiftly disruptive. Small amounts of metabolic inhibitors influence the osmotic behaviour of lutoids (GOMEZ, 1968).

The dilution reaction following tapping will alter the osmotic concentration of the latex and might subject the lutoids to some degree of osmotic shock. The effects of the dilution reaction on the composition of latex as collected have been much studied.

The dilution reaction causes a decline in rubber content of successive samples of latex collected (GOODING, 1952a), there is usually a recovery of concentration towards the end of flow (BOATMAN, 1966). The dilution recorded for the first drops of latex coming out may be affected by admixture with contents of cells other than latex vessels (BOATMAN, 1966).

FERRAND (1941) developed a method of collecting drop samples from the latex vessels which avoids most of the dilution effects attributable to tapping. DE JONGE (1955) made slight modifications to the method and used it to investigate the area of bark drained by flow. BOATMAN (1966) using de Jonge's technique investigated the changes in total solids content of latex at different positions on the latex vessels after tapping stimulated and unstimulated trees. Dilutions recorded during flow from a tree stimulated with 2,4,5-T (which reduces plugging) showed a gradient of total solids content extending over 25 inches from the cut, the sample taken nearest the cut (5 inches away) showing the greatest dilution effect. Relatively large gradients of total solids content can also exist in the neighbourhood of the tapping cut in the morning **before** tapping. These would modify the dilution effect as observed after

tapping. It is unsafe to rely on changes in total solids or rubber content during flow as a measure of the entry of water from the surrounding cells (BOATMAN, 1966).

Osmotic concentrations of successive flow fractions follow somewhat the same pattern as changes in total solids or dry rubber content, but again do not necessarily give a reliable index of dilution of latex by water from surrounding cells because the possibility of changes due to entry or release of solutes must also be taken into account (BOATMAN, 1966).

PAKIANATHAN (1967) determined the osmolarity of latex in the vessels from drop samples as 0.45 M. Rather higher values from other trees are quoted by PAKIANATHAN *et al.* (1966). After tapping the latex collected shows lower osmolarities. PAKIANATHAN *et al.* (1966) investigated 'bottom fraction' (mainly lutoids) damage in successive flow samples. In all cases damage scores were highest in the early flow fractions. This was shown not to be due to bacterial action, the authors attribute it to osmotic shock effects. The lowest osmotic concentrations recorded were not such as would be expected to damage lutoids to the extent observed, the authors therefore suggest that the damage would be accentuated by the rapidity of the change in osmotic conditions between the vessel and the collecting cup. The possibility that 'bottom fraction' damage in the early flow fractions might be due to factors other than bacterial effects or osmotic shock also needs consideration. All agencies which might cause damage to lutoids within the vessels are likely to be operative at greatest intensity during the early stages of flow. Osmotic shock may be only one factor. In addition latex collected during the early stages of flow may be modified by reactions along the cut, where it would be mixed with the contents of cells broken by tapping.

Osmotic changes in latex after collection do not necessarily correspond to changes occurring in the vessel. PAKIANATHAN *et al.* (1966) remark that much of the 'bottom fraction' damage observed must have occurred after the latex had left the vessel. Microscopy (SOUTHORN, 1968a) strongly suggests that lutoids suffer heavy casualties in their journey along the tapping cut. There is as yet little experimental information on osmotic concentration gradients in the vessel after tapping and it is not known whether these would be enough to disrupt lutoids within the vessels after tapping. Unless there is localisation of osmotic change near the ends of the vessels then there are difficulties in accepting osmotic shock alone as a cause of internal vessel plugging. It has been suggested that lutoids might be damaged for some distance below the cut but are swept out during the initial period of fast flow, remaining behind to plug the vessel only when flow rate falls (CHUA, 1965; PAKIANATHAN *et al.*, 1966; MILFORD *et al.*, 1969). This suggestion is tenable only if plugging does not in fact occur during fast flow.

It seems fairly certain that osmotic changes in the vessel after tapping would be in the general sense of assisting lutoid damage, but the extent to which osmotic shock alone is responsible for vessel plugging is still speculative. It is now well established that a sufficient degree of osmotic shock will disrupt lutoids, that collected latex shows a lower osmotic concentration than latex in the tree, and that first runnings of latex contain more damaged lutoids than the later flow. There is still uncertainty as to the extent to which lutoid damage in collected latex might be due to factors other than osmotic shock or whether osmotic changes in the vessels are sufficient to disrupt lutoids.

Mechanical Effects (Shear)

Optical and electron micrographs of latex vessels indicate that distortion of particles along flow lines can occur so that shear stresses could be important (SOUTHORN, 1968b). The flow properties of fresh latex are greatly modified by its 'bottom fraction' particles, particularly lutoids (VAN GILS, 1949, 1951a and b; RESING, 1959; VERHAAR, 1952 and 1954). When fresh latex is forced through glass capillaries with internal diameters approximating to those of latex vessels, effects are noted depending on the pressure gradients

employed. At moderate pressure gradients (up to 0.2 atm/mm) the flow rate is directly proportional to the pressure gradient, but the apparent viscosity decreases with the capillary diameter (SOUTHORN AND YIP, 1968b). In fact fresh latex flows appreciably more easily through a capillary of latex vessel dimensions than would be expected from applying Poiseulles law to quoted values for fresh latex viscosity. Very similar effects have been reported for blood flow through narrow bore capillaries. At higher pressure gradients, lutoids begin to break down, microflocs of rubber begin to appear in the outflow and there is a drop in the zeta potential of the rubber particles in the outflow as compared with those entering the capillary, indicating release of B-serum.

In fifteen out of eighteen experiments at relatively high pressure gradients the capillary plugged and flow stopped abruptly *(Figure 1)* at gradients of 0.4 to 1.2 atm/mm (YIP AND SOUTHORN, 1968). The behaviour of latex freed from lutoids by a previous centrifugation was quite different and flow could be maintained indefinitely with no case of plugging in thirty-nine experiments, in some cases extending to 13 atm/mm pressure gradient. The implications are that lutoids affect the flow characteristics of latex in narrow bore capillaries, and at sufficiently high pressure gradients lutoids are broken by shear, after which plugging of the capillary and cessation of flow usually occurs abruptly. In translating these results to flow behaviour in latex vessels it must be pointed out that collected latex was used, which had first to be freed from microflocs by filtration, and which therefore differed to an unknown extent from latex in the vessel, and that the capillaries were of glass, so that the wall surface also differed from that of a latex vessel. Nevertheless when these results are set alongside the electron micrographs showing shear effects in the latex vessel, the evidence seems very strong that shear effects could be important in plugging. Shear effects would to some extent be located near the severed ends of the vessels, where the pressure gradients must be very high for a short period after flow. It has already been suggested that if small increases in lutoid membrane permeability arise from electrical wound responses, these would lead to increased adhesion between lutoids which would make them more susceptible to shear damage in the region near the wound where we would expect this to occur. Osmotic effects may also contribute to the situation by sensitising the lutoid membrane to damage by shear.

If shear is an important factor in vessel plugging, then plugging would depend to a great extent on the ability of the tree to maintain relatively high pressure gradients near the vessel ends for long enough to accomplish sufficient lutoid damage within the vessels. The faster the initial high pressure gradient disappears, the less effective this plugging mechanism would be. The extent of wounding should be important in this respect. For a very extensive wound, say a very long tapping cut, there must be a swift wholesale depletion of turgor over a considerable zone following the rapid loss of fluid which occurs. If only relatively few vessels are severed, there would be less difficulty in maintaining relatively high turgor gradients in them for a rather longer period. Long cuts should therefore show a much smaller plugging intensity than short ones. Plugging is less noticeable after full-spiral tapping than after half-spiral tapping (MILFORD *et al.*, 1969) and yield stimulation is usually more effective if the tapping intensity is not too high. This would fit very well with the idea that shear and turgor gradients are important. Some very recent experiments also lend weight to this conclusion, and will be discussed later.

Chemical Effects

It is now well known that the stability of lutoids, and indeed the stability of latex even without lutoids, is easily altered by chemical agencies. The difficulty in postulating a plugging mechanism based on chemical intervention is that we know little about whether there is transference of appropriate chemicals across the vessel wall. As regards coagulation on the cut there would be a good supply of coagulants released from cells damaged by the knife, so that coagulation there is easy to

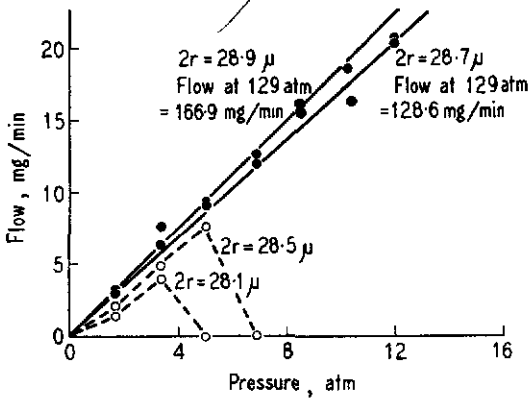

Figure 1. Flow of whole fresh latex and latex minus 'bottom fraction' through glass capillaries of 1 cm length under varying pressure (YIP AND SOUTHORN, 1968). ● *represents latex minus 'bottom fraction', and* ○ *represents fresh latex.*

understand in general terms, though the details of the coagulating mechanisms have not so far been settled. It is very difficult to believe that coagulants released along the cut could diffuse back into the vessels during the initial period of fast flow, though of course when flow had almost stopped, back diffusion would certainly take place consolidating whatever internal plugs had already formed. Indeed it is possible that internal plugging has a function in preventing excessive back diffusion of bark coagulants for too great a distance down the vessels. YIP AND SOUTHORN (1968) immersed the ends of very narrow bore glass capillaries, through which fresh latex was flowing, into coagulant solutions such as formic acid. In no case was it possible to stop flow by this means. Whether coagulants from damaged cells penetrate the latex vessel wall transversely near the ends is another matter. In many ways this seems unlikely but it is very difficult to devise experiments to put the matter to the test.

In another sense there is of course transport of chemicals in and out of the latex vessels whether flow is taking place or not. The latex vessel is not an isolated system: it exchanges materials with other cells as a component part of the whole organism. The question of biosynthetic activity, mode of action of yield stimulants, and indeed the whole life of the tree, is linked to these transfers.

YIELD STIMULATION

The relationship of the theoretical studies on lutoids to the yield stimulatory action of growth regulators is so far unclear. BLACKMAN (1961) discussed the physiology of yield stimulation and offered three alternative suggestions: that the internal pressure within the vessels was increased, that the properties governing flow within the vessels were changed, or that the severed ends did not plug so quickly. There has since been further work relevant to all these suggestions.

RUBBER RESEARCH INSTITUTE OF MALAYA (1964) has reported that increased turgor pressure in 2,4,5-T stimulated untapped trees, after tapping, the pressure fell lower than for untreated trees and remained lower for a longer time. The flow of course continues longer after stimulation (DE JONGE, 1953; CHAPMAN, 1951; HO AND PAARDEKOOPER, 1965; SCHWEIZER, 1953).

PAKIANATHAN *et al.* (1966) record osmolarities for latex over a number of tappings after 2,4,5-T stimulation which show no very obvious effect. BOATMAN (1966) found that after stimulation the osmotic pressure of latex was little influenced during the early part of flow, but rises in the later stages.

The idea that 2,4,5-T stimulation might increase flow by reducing latex viscosity was investigated by BOATMAN (1966) who could not detect any such effect.

The suggestion that growth regulators might reduce plugging of the vessel ends has turned out to be a very good one. We now know that 2,4,5-T reduces plugging intensity (BOATMAN, 1966); however we still do not know how this is accomplished. Latex from trees after stimulation contains many more damaged lutoids than does latex from the untreated trees (PAKIANATHAN *et al.*, 1966). This of course refers to latex as collected: we have no information about the effects of 2,4,5-T stimulation on lutoids within the vessels.

The number or size of latex vessels is not altered by yield stimulation (DE JONGE, 1957; GOMEZ, 1964). The extent of the zone of

bark affected by tapping is however enlarged. LUSTINEC *et al.* (1966) has distinguished three zones affected by tapping: the 'flow area' from which latex emerges, the 'displacement area' in which latex moves towards the cut during or shortly after tapping, and the 'equilibration area' in which slow changes can be detected for a considerable time. DE JONGE (1955) found that when 2,4,5-T stimulation produced large yield increases, these were accompanied by increases in the 'drainage area' (corresponding roughly to 'displacement area'). Similar conclusions are reached by LUSTINEC *et al.* (1967). Increased drainage area and withdrawal of more latex from the tree would bring about increased biosynthetic activity to replace losses. The possibility of this influencing the particle size distribution or the numerical ratio of lutoids to other particles may be worth considering (GOMEZ, 1966).

So far only the growth regulators such as 2,4-D or 2,4,5-T have been found suitable for commercial use, but other classes of chemicals have been shown to influence flow. In no case the mechanism is firmly established.

The stimulant action of copper has been much studied (COMPAGNON AND TIXIER, 1950; TIXIER, 1951; WIERSUM, 1953). Copper is an element required in many biosynthetic activities, but that copper stimulation acts by helping enzyme synthesis is not proven. A most interesting point is that 2,4,5-T stimulation brings about a detectable increase in the copper content of latex.

A spectacular response to ethylene oxide has been reported by TAYSUM (1961a). The tree died but it yielded latex continuously over several days. TAYSUM (1961b) also reports stimulant action from antibiotics applied in a bactericidal dressing containing alkaline detergent. Anionic surfactants are being tried in a different connection with the idea that they may improve the colloidal stability of latex within the vessels against B-serum release from lutoids (SOUTHORN AND YIP, 1968a). In one experiment in which solutions of surfactant were perfused into the bark, a strong positive effect was obtained, but this could not be duplicated in a later trial. Work is now in progress on chemical treatments which may modify membrane activity. Glutaraldehyde, a well known fixative, has so far consistently brought about large increases in flow time. Formaldehyde is thought to act similarly. BANCHI (1967) has reported a stimulatory action for chloroform.

PUSHPARAJAH (1966) has shown that if pads soaked with solutions of calcium salts are placed in contact with the bark below the cut, yield is significantly reduced. Calcium might perhaps alter membrane permeability, or augment the colloid destabilising activity of B-serum.

There has been interest in the possibility of influencing transpiration rates by metabolic inhibitors to improve yields (DECONINCK, 1965; NINANE, 1967). A variety of 'anti-transpirants' has been tried; some of them are reported to reduce the drop in yield which occurs during mid-day heat, and to show yield stimulatory effects.

PRESENT SITUATION AND PROSPECTS

It is fairly evident that physiological studies on latex flow have now entered a phase where there have been advances in theory which still have to be fully related to practice; but they have had a stimulatory effect on thought if not yet on commercial yield of rubber. A great many new lines of experimentation have opened up recently; some of these have already given important results. Since this is now such an active topic, I would like before I close to refer to some work still in its early stages and to offer a few personal speculations which I hope will soon be put to the test of experiment.

I have already referred to the effects on flow of altering the length of cut, a full-spiral cut usually giving a longer flow than a half-spiral cut (MILFORD *et al.*, 1969). In the light of the results on shear, studies are now in progress on the effects of wound size on plugging including very small cuts of one inch and less in length (SOUTHORN AND GOMEZ, 1968) *(Figure 2)*. As anticipated, the shorter the cut, the more dramatic is the plugging.

This work leads to the thought that *Hevea brasiliensis* has two distinct mechanisms

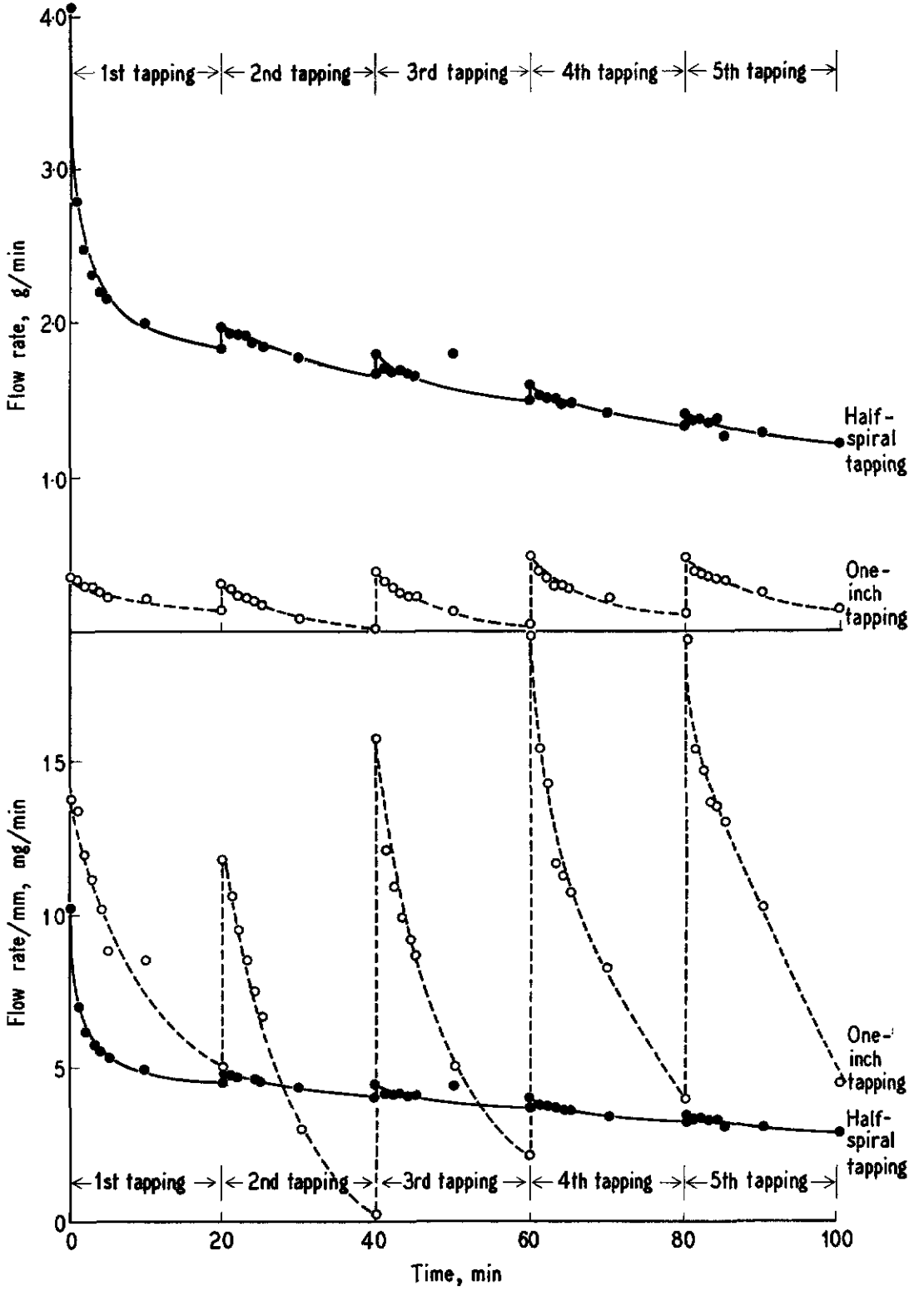

Figure 2. Flow curves for repeated opening of a half-spiral cut and an one-inch cut, expressed as total flow rate (upper curves) and flow rate per mm of cut (lower curves). There is a much higher intensity of plugging from the shorter cut (SOUTHORN AND GOMEZ, 1968).

for preventing excessive loss of latex on wounding. The first is internal plugging of the vessels probably brought about by shear assisted by osmotic and other effects. This plugging reaction is displayed with maximum effect for very small wounds, which would be the most frequent contingency the tree must guard against in its native state. For extensive wounds, such as we inflict when exploiting the tree by very long tapping cuts, this plugging reaction would be largely inoperative for reasons already given, and the tree then has to fall back on a second reserve mechanism whereby the latex flow in individual vessels falls to a low rate due mainly to widespread loss of turgor. At this point coagulation on the cut and coagulants diffusing from the cut bring flow to a stop.

There are clonal differences in plugging index as measured for a half-spiral cut (MILFORD *et al.*, 1969; PAARDEKOOPER AND SAMOSORN, 1969). Some clones such as RRIM 501 have shown only very slight plugging and response to yield stimulation in Malaysia, though their behaviour seems rather variable. The present concept of plugging index has already proved its worth but needs to be modified by the ideas just outlined. If these are correct it could be predicted that all trees of all clones would show a high intensity of plugging if the wound is reduced sufficiently in size. Probably for every tree there is also some length of tapping cut beyond which plugging becomes only a minor effect. This is most likely to be a clonal matter, but one subject to modification by all variables influencing turgor, such as climatic and soil conditions, transpiration rates and so on.

In conclusion let us take a final look at the prospects for application of recent studies on vessel plugging. They already give a new insight into flow behaviour and should eventually lead to a better control of exploitation of different clones in terms of use of known yield stimulants or variations in tapping intensity. They may well lead to introduction of new classes of yield stimulants. Beyond this, forecasts are increasingly speculative. There could be two objectives in eliminating vessel plugging. The first is to increase yield by prolonging flow from conventional tapping, an objective already attainable for many clones by the use of known yield stimulants and known tapping methods. The second would be to decrease costs by inducing flow for many days at a time, so that tapping could be less frequent. If we think of aiming at this second objective of very prolonged flow through elimination of plugging, the chances of success are small if treatments are applied under conditions where plugging is relatively unimportant. The first step towards success may come, if we can find ways of eliminating plugging for small tapping wounds where plugging seems to be a very major flow factor. There seems to be nothing in the present work so far to indicate that such is theoretically impossible.

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DISCUSSION

Chairman: Prof. G. E. Blackman

Prof. F. Lynen asked if sucrose density centrifugation had been tried to isolate the lutoid fraction and to investigate if it was heterogeneous. Dr. B.L. Archer said that a number of enzymes had been demonstrated in the lutoid fraction by Dr. S. Pujarniscle, using density-gradient centrifugation. Dr. Southorn replied that preliminary attempts had shown that there were difficulties in applying the sucrose density method to whole lutoids.

Prof. F. Lynen enquired if ATP-ase activity, which was either magnesium or calcium stimulated, had been detected in the membrane of the lutoid in connection with its supposed mechanism for active transport. Dr. J. B. Gomez said that the osmotic properties of lutoids isolated from collected latex were affected by metabolic inhibitors in the C-serum, which probably indicated active transport. Dr. Archer confirmed the presence of ATP-ase in the 'bottom fraction' of centrifuged latex: other degenerative enzymes such as phosphatase and lysozyme were present in the lutoid particles, which suggested that they were similar to lysozymes. Dr. Archer asked if the effects of oxygen and light on plug formation, and of a wettable surface on the stability of 'bottom fraction' (*cf.* blood coagulation) had been investigated. Dr. Southorn replied that his inability to give definite answers to these questions emphasised one of the major experimental difficulties involved in these investigations, namely, that the lutoids were available for study only after they had left the latex vessel when they had already changed (and were still changing), whereas these changes and plugging were probably initiated within the latex vessel.

Dr. G. Verhaar noted that lutoids appeared as large irregularly shaped bodies in fresh, unpreserved latex. He enquired what held them together, a membrane perhaps? Further, these bodies disintegrated into their units, if fresh latex was ammoniated: what was the effect of the ammonia on the membrane? Were the lutoids disrupted and their contents dispersed in the serum? What effect did this have on latex viscosity? Dr. Southorn replied that the first observations of lutoids were as aggregates. Miss J. Ruinen had later applied the term 'lutoid' to individual particles within these aggregates and this term was now used to refer to such an

individual particle bounded by a membrane. As the membranes lost polarisation and small cations moved from the inner to the outer surfaces, the zeta-potential of the lutoids and the electrostatic repulsion between them declined, allowing the lutoids to stick together. Thus, in latex as obtained from the tree, the lutoids had lost some of their ability to stay apart and were often seen as aggregates. The microviscometer provided the means to measure this 'sticking together' characteristic of the lutoids.

Dr. Southorn noted in passing that the last runnings of latex flow were better than the first for study of lutoids in their best condition. Lutoid membranes were exceedingly sensitive to changes in chemical environment, almost all of which accelerated the disintegration of the lutoids and the discharge of their contents into the latex. When ammonia was added as a dilute aqueous solution there were at least three effects: chemical, osmotic and pH. The first two would destabilise and disrupt the lutoids; the raising of the pH would improve the colloid stability of the latex and would tend to lower viscosity.

Mr. E. C. Paardekooper remarked that in experiments conducted by Dr. Verhaar as well as himself the addition of water alone increased the viscosity due perhaps to consequent swelling of the lutoids, whereas the viscosity fell on adding ammonia solution. He asked whether this was due to the lutoids being destroyed. Dr. Southorn agreed that controlled addition of moderate amounts of water caused swelling of the lutoids without breakage and markedly increased the apparent viscosity of the latex. On addition of ammonia, however, there was a drop in viscosity due partly to reduced aggregation of all particles as the pH began to rise and also because lutoids were broken and their contribution to viscosity was removed. Microflocs caused by release of lutoid contents had very little effect on the bulk viscosity.