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Changes in the Bottom Fraction Contents of Latex during Flow in Hevea brasiliensis

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Changes in the contents of bottom fraction of latex samples taken from the panel and the spout during latex flow have been followed by counting stained bottom fraction particles under the microscope. Results from normal, stimulated and repeatedly tapped trees support the suggestion that osmotic damage to bottom fraction is involved in latex vessel closure.

The flow of latex from *Hevea brasiliensis* is curtailed by the development of a blockage close to the cut ends of the latex vessels soon after tapping (BOATMAN, 1966). Electron microscopical studies of this region have shown the presence of both a cap of rubber coagulum over the cut ends of the latex vessels and plugs of coagulum within the vessels close to the severed ends; in both cases aggregated and damaged lutoids were associated with the rubber coagulum (SOUTHORN, 1968).

PAKIANATHAN *et al.* (1966) have shown that the osmotically sensitive bottom fraction is damaged by the reduction of osmotic concentration which occurs in the latex during flow. This leads to aggregation of lutoids and rubber particles, *i.e.* to the formation of flocs. SOUTHORN AND EDWIN (1968) have shown that the fluid contents of lutoids, which may be released after osmotic damage, have a powerful destabilising action upon rubber particles.

In latex collected after tapping, bottom fraction damage and flocculation were marked only in the latex fraction collected during the initial stages of flow despite the fact that equally low osmotic concentrations were encountered later. To account for this, PAKIANATHAN *et al.* (1966) suggested that the initial flow rates are sufficiently high to sweep the diluted latex out of the laticiferous system before irreversible damage to the bottom fraction occurs; but, later during flow, when there is little damage in the collected latex, the bottom fraction is damaged within the latex vessels forming 'flocs' which accumulate at the ends of the latex vessels thus initiating the sealing process.

If this suggested mechanism is correct, latex entering the collection vessel should contain fewer intact bottom fraction particles than latex within the latex vessels prior to exudation, particularly during the later stages of flow. In the experiments described in this paper we have attempted to establish this by counting the number of bottom fraction particles in small samples of latex taken from the panel and at the spout, at intervals, during flow from individual trees. The effects of stimulation and repeated tappings have also been examined.

MATERIALS AND METHODS

The experiments were carried out with fourteen and sixteen-year old budded trees of clone Tjir 1 grown in Field 47 of the Rubber Research Institute Experiment Station. The trees were tapped on a half spiral alternate daily on the second panel of virgin bark or on Panel C of renewed bark.

Application of Stimulant

The stimulants used in these experiments to increase latex yield were 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T), 2-chloroethyl-

phosphonic acid (Ethrel) and EDBROZA (an ethylene-generating system involving ethylene dibromide, zinc and ethanol).

2, 4, 5-T was applied as 1% acid equivalent incorporated in palm oil onto a 6.5 cm (2½ in.) band of scraped bark below the tapping cut. Ethrel (ABRAHAM *et al.*, 1968) was applied at 10% active ingredient in palm oil on a 4 cm (1½ in.) band of scraped bark below the tapping out. EDBROZA (PAKIANATHAN, 1970) was applied to a hole made by a drill just above the graft union below the tapping cut and a test tube containing 3.2 g of ethylene dibromide, 2.0 g of ethanol and 1.2 g of zinc was inserted into the bored hole. The hole was then sealed with tissue paper and latex concentrate.

Determination of Osmolarity of Latex and Solutions

Osmotic concentrations were determined with a Mechrolab Vapor Pressure Osmometer. The procedure has been described by PAKIANATHAN (1967).

Latex Sampling

Latex samples were taken from the tapping panel at positions 5 cm below the cut before tapping and at intervals during flow. Each of these samples of 0.25 ml was obtained by pooling five samples of 0.05 ml, which were extracted, by modification of DE JONGE'S (1955) *in situ* microsampling method, from points distributed beneath the length of the cut. At the same intervals, 0.25 ml samples of the latex dripping from the spout were taken.

Staining of Bottom Fraction Particles

A wide range of dyes belonging to the azo, quinone-amine and phenylmethane groups were tested for their specificity in staining bottom fraction particles. In addition observations were carried out to select the dye which caused minimum damage to bottom fraction particles.

About 250 ml of latex were collected under chilled conditions from one tree. To an aliquot of 7.5 ml of this latex, an equal volume of a phosphate buffer solution (0.025 M, pH 7.0) containing 0.02% of a dye, adjusted to an osmotic concentration of 500 mOsm *(hypertonic)* with potassium chloride was added. Similar mixtures were made with aliquots of 7.5 ml of latex, using the same osmotic concentration and buffer, but with the buffered osmoticum in each case containing 0.02% of a different dye. The mixtures in each case were gently stirred and transferred into centrifuge tubes and ultracentrifuged in a chilled No. 40 rotor (Spinco Model L ultracentrifuge) at 26 000 rev per minute for 45 minutes.

In another experiment the same procedure was followed using the same source of latex, but the final osmotic concentration was adjusted to 100 mOsm *(hypotonic)*. The concentrations of dye and buffer were 0.02% and 0.025 M, pH 7.0 respectively. The mixtures containing the various dyes were centrifuged as described above.

Determination of Bottom Fraction Counts

PAKIANATHAN *et al.* (1966) have shown that the irreversible damage that occurs to the bottom fraction can be alleviated by collection of the latex directly into hypertonic media, indicating that particle damage largely occurs after the latex has emerged from the cut ends of latex vessels. To prevent such damage, particle counts were made on latex samples collected into buffer-dye solutions which were slightly *hypertonic* to latex as it exists within the laticiferous system prior to tapping.

The samples of latex (0.25 ml) were collected into 24.75 ml of phosphate buffer (0.025 M, pH 7.0) adjusted to an osmotic concentration of 550 mOsm per litre with potassium chloride and containing 0.02% toluidine blue (vital). The latex was thus diluted a hundred fold and was subsequently diluted a further ten fold in the laboratory with the buffer-dye osmoticum before counting. The solutions were maintained at a temperature below 5°C at all stages until

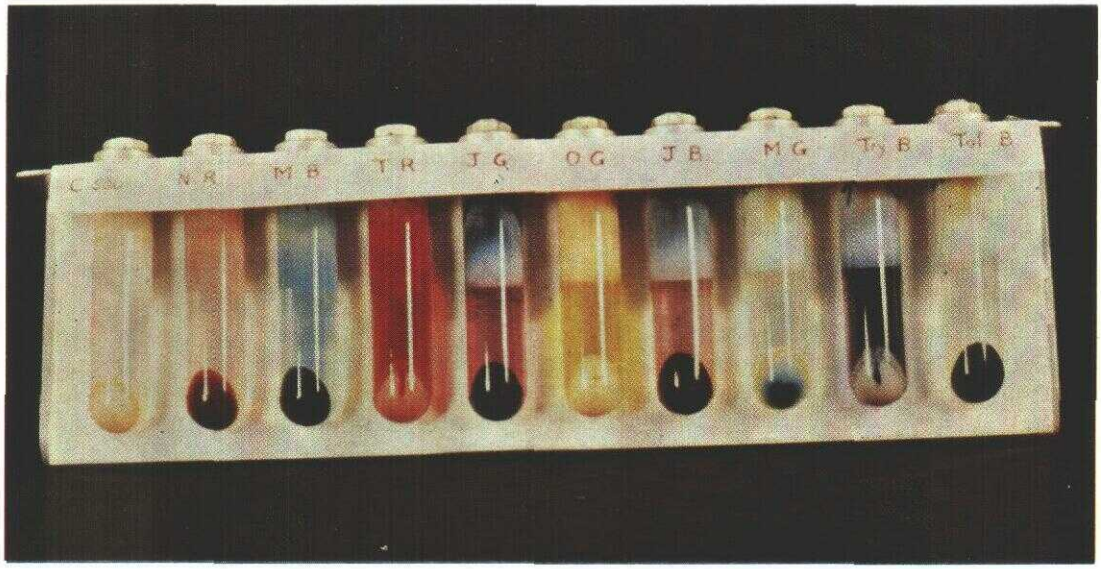
the actual counting. Under these conditions there was little evidence of osmotic damage since the bottom fraction particles were present as single units. The bottom fraction particles of sub-samples of the suspension were counted over twelve squares of a haemocytometer grid at × 400 magnification. The counts so obtained were adjusted for the dilution of the original sample which occurred as a result of latex dilution during flow. The extent of the dilution was determined from the fall of the osmolar concentration of latex samples taken at the same as the count samples from both the panel and spout positions.

RESULTS

Since the experimental technique involved the counting of stained bottom fraction particles under the microscope, it was necessary to show that toluidine blue (vital) was a suitably specific stain for bottom fraction particles and that the osmotic behaviour of the particles themselves was not affected by the concentration of dye used.

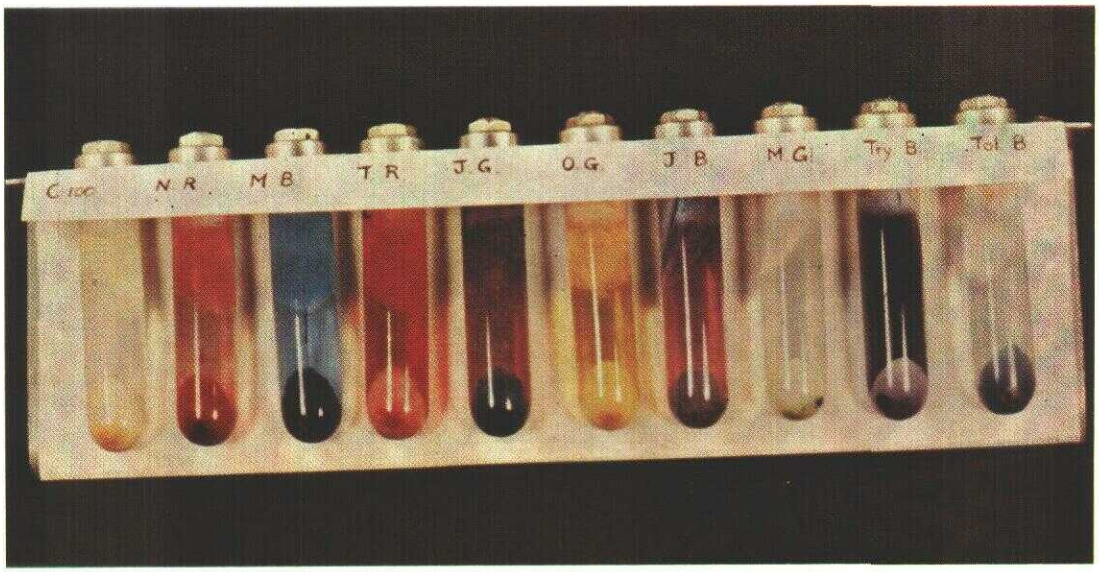
In *Figure 1*, it can be seen that among the dyes tested (*viz.* neutral red, methylene blue, trypan red, janus green, orange green, janus blue, malachite green, trypan blue and toluidine blue) the dye which only stained bottom fraction without staining the serum or latex particles was toluidine blue (vital). The dyes janus green, janus blue, methylene blue and neutral red stained both bottom fraction and the rubber particles just above the serum phase. The dyes trypan red, orange green and trypan blue failed to stain the bottom fraction particles. It is interesting to note that malachite green showed a different staining effect on the bottom fraction. None of the stains caused damage to the bottom fraction particles when compared with the unstained control.

All the samples subjected to the hypotonic (100 mOsm) medium showed damage. This can be seen by the irregular boundaries of the



C = Control. N.R. = Neutral red. M.B. = Methylene blue. T.R. = Trypan red. J.G. = Janus green. O.G. = Orange green. J.B. = Janus blue. M.G. = Malachite green. Try. B. = Trypan blue. Tol. B. = Toluidine blue.

Figure 1. Staining of bottom fraction in hypertonic solutions with various dyes.



C = Control. N.R. = Neutral red. M.B. = Methylene blue. T.R. = Trypan red. J.G. = Janus green. O.G. = Orange green. J.B. = Janus blue. M.G. = Malachite green. Try. B. = Trypan blue. Tol. B. = Toluidine blue.

Figure 2. Staining of bottom fraction in hypotonic solutions with various dyes.

bottom fractions of the samples *(Figure 2)*. The degree of damage by osmotic shock in the unstained bottom fraction sample was similar to that in the toluidine blue stained sample, indicating that the addition of dye at a concentration of 0.02% to the latex did not alter the physiological behaviour or cause further damage to the particles, and that the damage to bottom fraction particles observed after centrifugation was mainly due to a lowering of the osmotic concentrations rather than to the toxic effects of the dye itself.

Changes during Normal Latex Flow

The results presented in *Table 1* are from experiments carried out on three trees on separate occasions. Trees A and B yielded 74 and 77 ml of latex respectively and flow ceased soon after the final samples were taken. Tree C yielded 110 ml and flowed for a further period of 10 min after the final samples were taken. The osmolar concentrations of latex samples taken both from the panel and the spout showed typical dilution patterns in all the trees. There were, however, large differences between the extent of dilution at the two positions. A minimum osmotic concentration equivalent, which varied from 435 to 440 milliosmoles per litre, was observed by microsampling the panel compared with variations of 339–340 milliosmoles per litre in samples taken from the spout — a difference of approximately two atmospheres in osmotic pressure. In all trees, latex samples taken from the panel showed a slight decline during flow in the number of bottom fraction particles per millilitre of latex which could not be accounted for by dilution effects; whereas, the number of bottom fraction particles declined rapidly in latex collected at the spout. There was therefore a progressive loss of particles from the latex while it flowed

TABLE 1. FLOW RATES AND CHANGES IN BOTTOM FRACTION CONTENT AND OSMOLAR CONCENTRATIONS OF LATEX SAMPLES TAKEN FROM LATEX VESSELS AND TAPPING SPOUT DURING FLOW FROM THREE UNSTIMULATED TREES (A, B AND C)

Time from tapping (min)	Flow rate (ml/min)			Osmolar concentration of latex (milliosmoles/litre)						Bottom fraction particles per ml of latex ($\times 10^{-6}$)						Particles lost between latex vessels and collection spout (%)		
				Within latex vessels			At collection spout			Within latex vessels			At collection spout					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
−5	–	–	–	505	495	502	–	–	–	106.0	102.0	105.3	–	–	–	–	–	–
5	4.8	4.7	4.8	473	478	479	397	387	402	99.2	95.8	98.7	89.2	87.7	80.2	10.1	8.5	18.7
15	1.9	2.0	2.3	444	453	468	351	368	363	93.2	93.7	95.2	76.0	75.2	79.5	18.5	19.7	16.5
25	1.1	1.3	1.8	440	442	452	340	359	357	93.2	92.6	93.2	63.2	72.1	68.3	32.2	22.1	26.7
45	0.4	0.5	1.1	435	438	442	359	350	339	95.2	91.2	92.0	51.6	59.3	52.1	45.8	35.0	43.4
60	0.3	0.3	0.6	457	447	440	364	346	350	94.8	93.2	94.2	47.2	48.2	53.2	50.2	48.3	43.5
75	0.1	0.1	0.4	461	459	453	360	353	359	94.4	92.1	91.8	45.6	49.2	44.5	51.7	47.0	51.5
90	0.07	0.06	0.2[a]	465	463	459	361	355	366	93.6	93.7	94.5	46.0	47.1	48.2	5.09	49.7	4.90
Total yield (ml)	74	77.0	110															

[a] Flow ceased after a further 10 minutes.

TABLE 2. FLOW RATES AND CHANGES IN BOTTOM FRACTION CONTENT AND OSMOLAR CONCENTRATIONS OF LATEX SAMPLES TAKEN FROM LATEX VESSELS AND TAPPING SPOUT DURING FLOW AT THE FOURTH TAPPING AFTER STIMULATION OF THREE TREES (A, B AND C)

Time from tapping (min)	Flow rate (ml/min)			Osmolar concentration of latex (milliosmoles/litre)						Bottom fraction particles per ml of latex (× 10-6)						Particles lost between latex vessels and collection spout (%)		
				Within latex vessels			At collection spout			Within latex vessels			At collection spout					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
– 5	–	–	–	516	510	520	–	–	–	106.0	101.7	105.8	–	–	–	–	–	–
5	5.8	5.2	6.2	436	463	462	410	448	453	104.0	94.3	83.7	104 0	93.1	91.7	0	1.3	2.1
15	2 4	3.8	5.4	434	469	460	391	431	440	97 6	93.1	91.2	99.6	90.3	90 1	0	3.0	1.2
25	1.4	2.9	4.5	421	452	450	381	425	420	98.4	92.6	94.1	95.2	88.1	89.7	3.2	4.9	4.7
45	0.8	2.2	3.4	415	451	443	382	416	412	95 6	93.9	92.7	82.4	80.4	79.8	13.8	14.4	13.9
60	0.6	2.1	3.0	411	442	440	381	400	389	95.2	94.2	93.2	71.2	73.4	74.2	25.2	22.1	20.4
75	0.5	1.8	2.4	434	445	441	379	393	392	94.4	94.6	91.9	55.2	69.8	72 1	41.5	26.2	21.6
90	0.4[a]	1.3	2.7	440	447	449	374	387	386	94.0	93.7	94 0	49.2	63.7	70 2	47.7	32.0	25.3
150	–	1 1	2.1	–	439	452	–	396	397	–	92.4	92.3	–	56.3	64.1	–	39.1	30.6
210	–	0.9	1.2	–	441	453	–	392	412	–	94.2	93.4	–	52.1	60.3	–	44.7	35.4
270	–	0 5	1.0	–	453	448	–	389	410	–	93.1	92.9	–	51.4	57.5	–	44.8	38.2
390	–	0.1[a]	0.8[b]	–	449	443	–	400	402	–	93.2	93.6	–	50.1	54.3	–	46.2	42 0
Total yield (ml)	112	374	675															

[a] Flow ceased after a further 10 minutes.

[b] Flow ceased after a further 60 minutes.

down the tapping cut *en route* from the latex vessels to the collection vessel. This loss probably represents the accumulation of damaged particles either within the cut ends of the latex vessels or in the coagulum on the surface of the tapping cut. This accumulation commenced within 5 min of tapping and involved only 10% of the particles and progressively increased until, in the final few millilitres of latex collected, approximately half the bottom particles were lost.

Effect of Yield Stimulation

Table 2 shows the results from the fourth tapping after the same trees (A, B and C) were stimulated with 2, 4, 5-T, Ethrel and EDBROZA respectively. Both higher flow rates and longer flow times contributed to an increased yield with all three stimulants. EDBROZA and Ethrel-treated trees flowed for a much longer time than those treated with 2, 4, 5-T.

Observations on Tree A stimulated with 2, 4, 5-T were discontinued 90 min from tapping, but latex flow continued for a further 15 min giving a final yield of 130 millilitres. In the case of Trees B and C, stimulated with Ethrel and EDBROZA, samples were taken until 390 min from the time of tapping. In the Ethrel-treated tree (Tree B), flow ceased 10 min after the last recording and gave a final yield of 380 millilitres. The EDBROZA-treated tree (Tree C) flowed for a further 60 min after the last samples were taken and gave a final yield of 752 millilitres.

Typical dilution patterns in the osmolar concentrations of the latex were again observed, together with marked differences in the extent of dilution at the panel and spout. The fall in osmolar concentration in latex samples taken at the panel was much greater following stimulation, suggesting an enhanced influx of water into the vessels during tapping. In spite of the markedly prolonged flow obtained, especially with Ethrel- and EDBROZA-treated trees, the osmolar concentration of latex in the panel did not fall below 440 mOsm per litre. This indicates that perhaps mineral ions and other osmotically active constituents may be counter-acting the dilution effect.

The changes during flow in the numbers of bottom fraction particles in the panel samples were essentially similar to those observed prior to stimulation. However, there was no significant decline in the bottom fraction particle count of latex samples collected at the spout till 45 min from tapping, indicating that stimulation delayed particle accumulation within the cut ends of the latex vessels or in the coagulum on the surface of the cut.

Effect of Repeated Tapping

BOATMAN (1966) has shown that plugs developing during flow can be removed by reopening the cut at frequent intervals while latex flow is still occurring, causing a sharp increase in the flow rate which then declines to a low level. The effects of reopening the tapping cut 30 min after the initial tapping on flow rates, latex osmolar concentrations and bottom fraction particle counts are shown in *Table 3*. During the first 30 min following the initial tapping, the changes in these parameters were essentially similar to those described for the unstimulated tree. Reopening of the cut resulted in a characteristic resurgence of latex flow and induced only a slight lowering of the prevailing osmolar concentrations in latex collected both from the panel and at the spout. Furthermore, reopening had little effect upon the number of bottom fraction particles in latex samples taken from the panel as compared with the preceding flow. However, the number of bottom fraction particles in the latex collected at the spout rose markedly in samples taken within 5 min of the repeated tapping before declining again during subsequent flow; the pattern of flow was similar to that of the initial tapping.

DISCUSSION

In these experiments latex samples collected directly into *hypertonic* solutions within the

TABLE 3. EFFECT OF REPEATED TAPPING UPON FLOW RATES AND CHANGES IN BOTTOM FRACTION CONTENT AND OSMOLAR CONCENTRATIONS OF LATEX SAMPLES TAKEN FROM LATEX VESSELS AND TAPPING SPOUT DURING FLOW

Time from tapping (min)	Flow rate (ml/min)	Osmolar concentration of latex (milliosmoles/litre)		Bottom fraction particles per ml of latex ($\times 10^{-6}$)		Particles lost between latex vessels and collection spout (%)
		Within latex vessels	At collection spout	Within latex vessels	At collection spout	
−5	–	520	–	104.0	–	–
5	4.0	444	416	103.6	75.2	27.4
15	1.8	447	338	100.0	60.0	40.0
30	0.7	460	336	101.2	46.4	54.2
Repeated tapping						
1	4.0	440	332	103.2	98.0	5.0
5	3.1	411	331	102.4	98.8	3.5
15	2.3	446	321	100.4	71.6	28.9
30	0.7	449	310	100.8	65.2	35.3

first 15 min after tapping showed a loss of only 10 – 20% of the particles, whereas the later fractions showed a more significant loss. Ultracentrifugation of successive flow fractions of latex showed that the initial flow fractions were more damaged than the later fractions. However, this damage was largely corrected when the initial flow fractions were collected directly into cold hypertonic buffered solutions (PAKIANATHAN *et al.*, 1966). Thus, the discrepancy between observations made by the counting technique and by ultracentrifugation studies only arose when the samples were not collected in hypertonic solutions. It appears that the bottom fraction particles from the initial flow fraction must have been sensitised by osmotic shock and shear forces operative during the movement of latex from the vessels and its subsequent emergence out of the partially collapsed latex vessels onto the cut. These particles were rapidly swept away from the cut due to the initial high flow rates. Collection of early-flow fractions into hypertonic solutions prevented rupture of the particles. Ultracentrifugation of initial samples of latex in the absence of a hypertonic medium caused more damage to the sensitised bottom fraction particles because of further shear forces introduced by ultracentrifugation at 26 000 rev per minute. Thus, the counting method provides a more accurate estimate of the condition of the particles at the time of collection of latex soon after the tapping operation.

The results reported in this paper show that the accumulation of damaged particles within the latex vessels or in the coagulum formed on the surface of the cut is a probable cause of the losses of bottom fraction particles from the latex while it is flowing from the latex vessels to the collection spout. In the comparatively low-yielding Tjir 1 trees this accumulation of particles commenced within a few minutes of tapping and was consolidated as flow progressed. Application of 2, 4, 5-T, Ethrel and EDBROZA delayed accumulation, but whether this was an in-

TABLE 4. OSMOLAR CONCENTRATIONS (MILLIOSMOLES PER LITRE) OF LATEX SAMPLES TAKEN FROM LATEX VESSELS AT VARIOUS DISTANCES BENEATH TAPPING CUT AT INTERVALS DURING FLOW

Distance beneath tapping cut (cm)	Time from tapping (min)			
	−5	5	15	30
5	540	434	469	519
30	537	418	473	486
60	559	446	496	507

Flow ceased at 32 min from tapping

direct consequence of an effect of the treatment upon flow rates or due to a direct effect upon factors causing particle flocculation remains to be determined. The excision of a further millimetre or so of bark by repeated tapping removes both the developing plugs within the latex vessels and the coagulum cap, thus providing a clear path for the resumption of flow.

The results support the suggestions made by PAKIANATHAN *et al.* (1966) to account for their observations upon bottom fraction damage during normal and stimulated flow. However, repeated tappings indicate that the plugging process occurs within the millimetre of latex vessel close to the tapping cut. This localisation of latex vessel closure must be adequately explained. Furthermore, dilutions within the laticiferous system persist throughout the period of latex flow at positions relatively remote from the tapping cut *(Table 4)*. If osmotic damage to bottom fraction occurs within the latex vessel when the flow rates have declined as originally suggested by PAKIANATHAN *et al.* (1966), why is it that plugs do not develop throughout the drainage area and cause the trees to cease yielding altogether? The osmolarity of latex within the vessels has not been observed to fall below 400 mOsm *(Tables 1* and *2)*. At this value only sensitisation of the particles would be expected to occur with very little or no damage to the particles.

In the experiments the most likely site of accumulation of the majority of bottom fraction particles lost between the latex vessels and the tapping spout is within the coagulum cap as the latex moves down the cut. Large islands of coagulum with the characteristic yellow appearance of the bottom fraction have been observed in this position. Little evidence has been obtained of gross damage to bottom fraction within the latex vessels during flow, but the collection of latex into hypertonic medium for counting may have prevented detection of this.

It has also been observed that osmotic concentrations during flow are consistently lower in latex that has left the tree than in samples taken from the panel. Osmotic damage to bottom fraction is therefore likely to be greater on the surface of the tapping cut than within the latex vessels. Consequently, latex vessel closure may be largely an external phenomenon. These findings also indicate the importance of collecting flow fractions into hypertonic media and the variations in number and properties of particles which could exist between successive flow fractions.

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