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Preliminary Physiological Studies on the Promotion of Latex Flow by Plant Growth Regulators

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Some mechanisms whereby plant growth regulators promote latex flow have been investigated. It is unlikely that any major part of the effect is attributable to changes in latex vessel collapse or in latex viscosity. Repeated reopening of the tapping cut has demonstrated that flow may be restricted rather rapidly by some process occurring at or near the surface of the cut; this process is apparently delayed following 2,4,5-T treatment. There is no clear evidence in favour of an effect on osmotic and turgor pressures or on the entry of water into the latex vessels during flow, but measurement of these quantities is subject to various complications in interpretation. There is some indication of a lowering of rubber content after treatment with 2,4,5-T. Such a fall cannot however be entirely explained in terms of the increased losses from the tree due to increased yields, and it is not experienced in rested trees treated with the growth regulator. It seems unlikely that the process affected by 2,4,5-T involves bacterial contamination of the cut ends of the latex vessels.

The promotion of latex flow by 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been known for many years (CHAPMAN, 1951; BAPTIST AND DE JONGE, 1955) and these two compounds are now widely used for this purpose. Attempts to elucidate the mode of action of such substances are discussed here.

Informal discussions during the Natural Rubber Research Conference held in Kuala Lumpur in 1960 indicated the following possible mechanisms by which plant growth regulators might increase latex flow:

(1) Application of a growth regulator to the bark near the tapping cut led to a rise in osmotic and turgor pressures, and a higher rate of flow on tapping.

(2) Flow was promoted by a lowering of latex viscosity.

(3) Flow was increased by changes in the elasticity of the latex vessel walls. There are two contrasting opinions on this mechanism:

- (a) The latex vessels show a greater degree of collapse on tapping following growth regulator treatment, thereby expelling a larger proportion of their contents.
- (b) The collapse of the latex vessels near the tapping cut is a major obstacle to continued flow and that the amount of collapse is reduced by the 2,4-D or 2,4,5-T treatment.

(4) After treatment, changes in membrane permeabilities permitted a greater inflow of water and solutes into the latex vessels during flow (along turgor and concentration gradients) and that such inflow permitted a greater discharge of latex.

(5) Flow is normally restricted by a process of sealing or coagulation at or near the cut surface and the 2,4-D or 2,4,5-T delayed this, possibly by an improvement in latex stability or by direct or indirect effects on destabilising micro-organisms.

The study was made with these suggestions in mind.

MATERIALS AND METHODS

The work was carried out on trees of clone Tjir 1 in Field 47 of the R.R.I.M. Experiment

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Station, budded in 1951 and tapped alternate-daily on a half-spiral cut (S/2.d/2.100%) in virgin bark of the first tapping panel. Such trees are, in fact, somewhat young for maximum response to growth regulator treatment, but do not have the disadvantage, as in older trees, of latex vessel discontinuities due to bark of different ages on opposite sides.

The growth regulator employed consisted of one percent acid equivalent of the n-butyl ester of 2,4,5-T in a carrier of palm oil and petrolatum (5:3 by weight). The mixture was normally applied to a strip of lightly scraped bark, 2.5 inches in width, immediately below the tapping cut. Control trees usually received a scraping and carrier treatment.

Yields were recorded by measurement of latex volumes and estimation of total solids and dry rubber contents. Flow rates were recorded in several experiments by timed collection into calibrated containers. For certain observations (e.g. freezing point determinations) the collecting vessel was surrounded by crushed ice contained in a vacuum jar and during subsequent handling the latex was maintained at temperatures below 5°C. Specific procedures for individual experiments are described separately.

RESULTS

Decrease in Trunk Diameter after Tapping

PYKE (1941), using a sensitive dendrometer, observed a rapid decrease in trunk diameter of about 30 μ immediately below the tapping cut when it was re-opened. GOODING (1952a) also observed contractions, of about 20 μ, one inch below the tapping cut using a slight modification of Pyke's instrument (GOODING, 1952b). If the rapid decrease in trunk diameter is due to the collapse of latex vessels beneath the cut, changes in this phenomenon following 2,4,5-T treatment might be detected by measurements at a series of tappings before and after treatment.

Making measurements with a re-constructed version of one of Gooding's dendrometers one inch below the tapping cut, an immediate difficulty was encountered with the large variation in the extent of contraction from day to day. This was thought to be due to variations in the depth of tapping and hence the number of latex vessel rings opened. There is usually some variation from day to day and, on the same day, between different parts of the cut, and it seems probable that such variation was exaggerated by the care required to avoid disturbing the delicate dendrometer close under the cut. No satisfactory solution to this problem was found, but sufficient observations were taken for any possible trends due to 2,4,5-T treatment to appear.

Results of a typical series of records on a single Tjir 1 tree are summarised in *Table 1.* The decrease in trunk diameter five minutes after tapping varied 38–63 μ before 2,4,5-T treatment and 38–61 μ after treatment. Anatomical observations on samples taken after the last experimental tapping suggested that the number of latex vessel rings opened had varied between 9 and 13, which could account for much of the variation. Taking a mean figure of eleven rows of latex vessels opened, and assuming that collapse of surrounding cells was negligible at five minutes after tapping, the average decrease in latex vessel diameter has been estimated (*Table 1*). Measurements on sections after fixation, which probably approximate to the dimensions at full turgidity, gave a mean diameter for the latex vessels in the outer eleven rows of 22 μ. According to the estimated contractions, this would decrease to 16.3–18.5 μ, after tapping—not sufficient to stop the flow, although some reduction in flow rate might be expected. No clear effect of 2,4,5-T treatment on contraction was noted either in this experiment or in others.

Contractions after tapping observed during the present work (30–70 μ per trunk diameter) have been consistently higher than those recorded by Pyke and Gooding, perhaps largely due to differences in the trees employed.

Changes in Flow Rate, Total Solids Content and Viscosity of Latex

For the rapid measurement of viscosity on small latex samples, a small viscometer was

TABLE 1. LATEX YIELDS AND TRUNK CONTRACTION

(Trunk contraction measured five minutes after tapping, one inch below the cut. An average of 11 rows of latex vessels were opened)

Period	Tapping number	Latex yield ml	Trunk contraction μ	Estimated decrease in latex vessel diameter μ
	1	20	38	3.5
	2	33	63	5.7
Before	3	25	43	3.9
treatment	4	26	48	4.4
	Mean	26	48	4.4
	5	40	61	5.5
After	6	55	61	5.5
2,4,5-T	7	91	58	5.3
treatment	8	87	38	3.5
	9	135	41	3.7
	Mean	82	52	4.7

specially constructed. The instrument operates on a modification of the rotating concentric cylinder principle, in which the sample container revolves on a gramophone turntable with a stainless steel cylinder immersed in the sample and suspended by a torsion wire. The angle of displacement of a pointer attached to the top of the cylinder was measured at each of the four operating speeds (16, 33, 45 and 78 rev/min), thus covering a range of shear rates. The apparatus was calibrated with liquid paraffin/kerosine mixtures of known viscosity. A sample size of 12 ml was usually employed at an operating temperature of 25°C.

Viscosity and total solids content were measured on aliquots of successive 20 ml samples taken during flow. Flow rates were also recorded. Two typical flow curves are given for Tjir 1 trees in *Figure 1*. As anticipated, the observed viscosity is heavily dependent on the rate of shear, particularly in the early samples after tapping, but the general form of the changes is similar at all four speeds. There is some correlation between viscosity and total solids content but this is by no means complete. Thus the last sample in (A) shows recovery in total solids but a fall in viscosity, and although the viscosity of each sample from Tree (B) is lower than the comparable one from (A), the ranges encountered during flow overlap, whereas those of the total solids contents do not. The total solids content may be taken, in this context, as a rough measurement of rubber content (which is usually about three percent lower than the total solids value in latex from these Tjir 1 trees). If the observed viscosity is heavily dependent on the non-rubber lutoid particles (Verhaar, 1956), no close relationship can perhaps be expected between it and the rubber content.

It will be noted that the flow rate of the initial sample was higher in (A) than in (B), despite the lower viscosity of the latter. (The lengths of the tapping cuts on the two trees were similar).

Figure 2 gives data for a single tree before and after treatment with 2,4,5-T. For simplicity only viscosities measured at 45 rev/min are given; similar trends were observed at the other speeds. Following 2,4,5-T treatment there was a marked tendency for samples collected immediately after tapping to show rapid partial coagulation, rendering viscosity and total solids determinations unreliable. Thus measurements for the first sample after treatment must be regarded with reserve. Before treatment, total solids content and viscosity follow a similar pattern, falling during flow and recovering at the last stages. The flow rate declines steadily. Following treatment, the flow rate is higher during the first 20 ml and, in the later stages, falls much less rapidly.

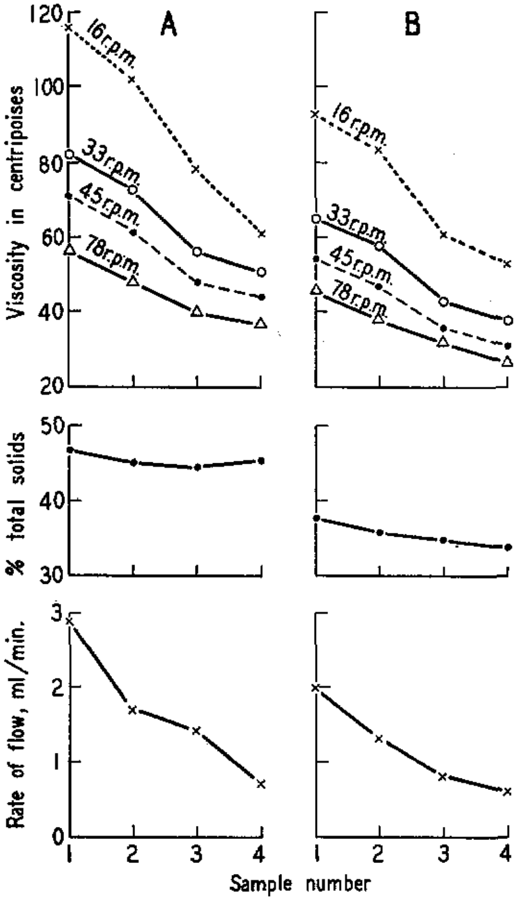


Figure 1. Viscosity, as measured at four rates of shear, total solids content and rate of flow for successive 20 ml samples of latex collected after tapping from two Tjir 1 trees, A and B.

The total solids contents, which are all well below those before treatment, show a small fall which becomes very slight as flow proceeds. Viscosity declines until the third sample and then fluctuates. There is no evidence of a decrease in viscosity due to treatment.

Trends at successive tappings before and after 2,4,5-T treatment are shown in *Figure 3.* Again, only viscosities recorded at the 45 rev/min speed are quoted. Latex yields varied between 60 and 90 ml before and 130–350 ml after treatment. Comparisons were therefore made, on the mean viscosity for the first 60 ml, which should be comparable for all tappings, and also over the whole flow. Total solids contents were compared on the same basis. Considerable fluctuations in viscosity occurred from day to day before treatment but these did not seem to be correlated with trends in yield or total solids. There was an apparent drop in viscosity, especially in the first 60 ml, immediately after 2,4,5-T application, but it had returned to normal levels by the time the highest yields were recorded. Again there

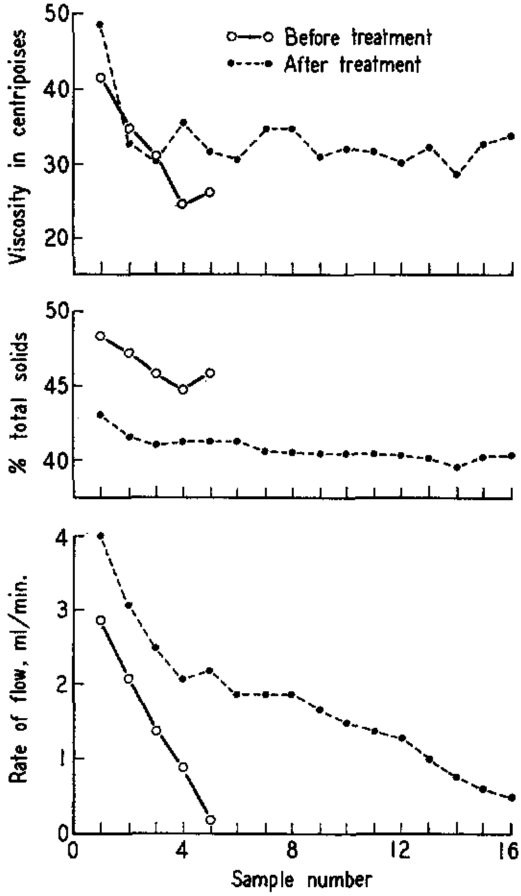


Figure 2. Viscosity, measured at 45 rev/min total solids content and rate of flow for successive 20 ml samples of latex collected after tapping both before and after treatment of a Tjir 1 tree with 2,4,5-T.

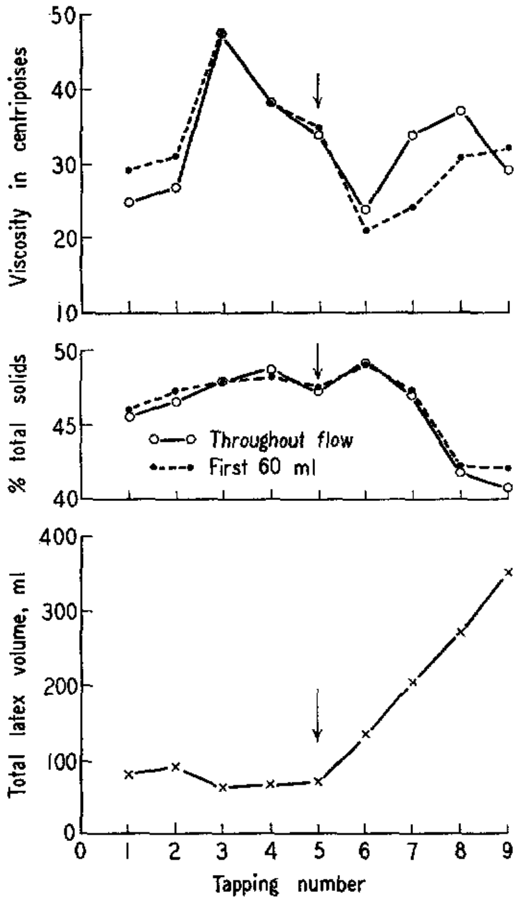


Figure 3. Trends in viscosity, measured at 45 rev/min and total solids content of latex for either the first 60 ml collected after tapping (●—●) or throughout flow (O—O), before and after treatment of a Tjir 1 tree with 2,4,5-T. The treatment was applied after the fifth recorded tapping, as indicated by the arrows. Total latex yields (×—×) are included for comparison.

appeared to be little correlation with total solids contents. A control tree, scraped and treated with carrier, showed only small changes, within normal variation.

From these results it was concluded that it was unlikely that major changes in latex viscosity were associated with the increased yields obtained following 2,4,5-T treatment.

Trends in Latex Flow Rates after 2,4,5-T Treatment

DE JONGE (1955) reported an increase in rate and duration of latex flow following 2,4,5-T treatment and a similar result was shown in *Figure 2*. Closer examination of changes from day to day (*Figure 4A*) shows that, although an increase in initial flow rate frequently follows treatment, this increase may

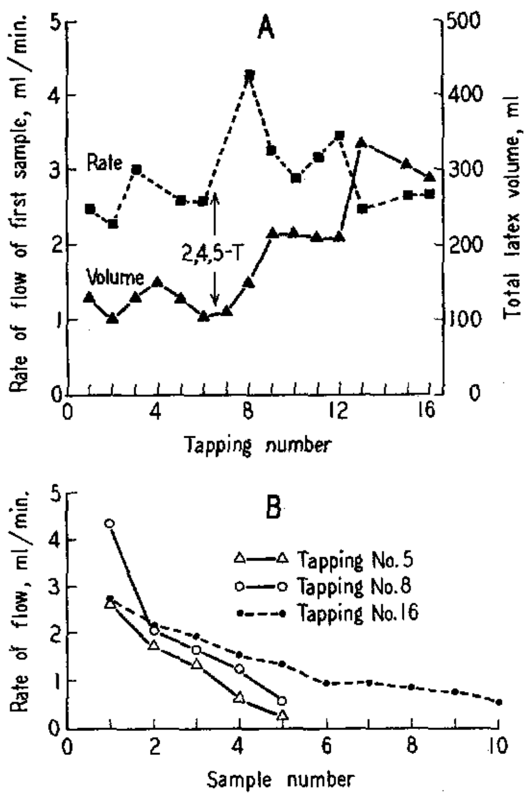


Figure 4. Effect of 2,4,5-T treatment on latex yield and rate of flow of a Tjir 1 tree.

A. Trends in rate of flow for the first 30 ml of latex collected after tapping and the total volume finally collected, before and after 2,4,5-T treatment (which was applied after the sixth recorded tapping).

B. Changes in rate of flow for successive 30 ml latex samples collected (No. 5); soon after treatment (No. 8); and during the period of peak yields (No. 16).

not persist, even though total latex yields continue to increase. Changes in rate during flow are examined in *Figure 4B* for three selected tappings: one before treatment, one shortly after treatment, showing an increase in initial flow rate, and one later, with a normal initial rate of flow but greatly extended duration of flow. The form of the curves on the two occasions after treatment is so clearly different that this might suggest that two different mechanisms may be operating, one predominating in the early stages and the other later.

Dilution during Latex Flow

It is well-established that, during flow, water enters the latex vessels from the surrounding tissues due to the large gradient in turgor pressure, and this results in a decline in dry rubber and/or total solids content of successive samples of the latex collected (GOODING, 1952a). Some recovery in concentration is usually noted towards the end of flow, as in *Figure 2* before treatment. After treatment the general level of rubber or total solids content is lower, but there is surprisingly little dilution during flow considering its prolonged duration. Further observations on trends both in total solids content and osmotic pressure (as determined by freezing point) are presented in *Figure 5*, which refers to the same experiment and the same tappings as in *Figure 4*.

Before treatment a normal fall in total solids content and osmotic pressure followed by partial recovery are recorded for both measurements. The high rate of flow soon after 2,4,5-T treatment is seen to be accompanied by an unusually low total solids content. Later samples show a tendency to rise rather than decrease in content. This contrasts sharply with the essentially normal pattern shown by the osmotic pressure measurements. At the later stage of response to 2,4,5-T treatment, the total solids content of the initial sample is even lower (despite the normal flow rate) and, after a small decline, there is a tendency to increase from the fourth sample onwards. The freezing-point determinations show a fairly normal initial osmotic pressure which declines

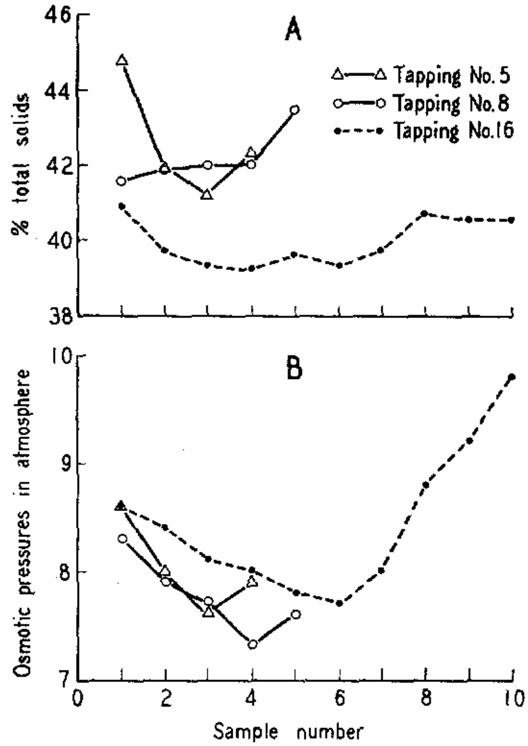


Figure 5. Effect of 2,4,5-T treatment on changes in total solids content (A) and osmotic pressure (B) of successive 30 ml samples of latex collected after tapping. Data from the same experiment and the same tappings as in Figure 4.

rather slowly until the fifth sample and thereafter rises markedly to higher levels than first observed.

Diurnal changes in turgor pressure of the laticiferous system in the trunk of untapped *Hevea* trees have been attributed to a loss of water to the xylem, which is thought to be under transpirational stress during daylight hours (BUTTERY AND BOATMAN, 1964). Clearly the later in the day flow continues, the greater will the tendency be for a reconcentration of latex due to transpiration effects. The prolonged flow in treated trees would particularly exhibit this phenomenon. While this might explain a tendency for latex concentration to rise towards the end of flow in treated trees, it can hardly account for the tendency for latex concentration to rise soon after tapping, as noted in some experiments. Neither can it explain discrepancies between trends in total solids contents and osmotic pressures.

The tendency for the osmotic pressure to increase sharply during the latter half of flow was observed in several trees after 2,4,5-T treatment. Thus, in *Table 2*, osmotic pressures of the first and last 30 ml samples from another experiment are compared. Treatment has little effect on initial osmotic pressure, but

TABLE 2. OSMOTIC PRESSURES OF FIRST AND LAST LATEX SAMPLES DURING FLOW

(Means of five tappings before and eight after treatment)

Sample	Osmotic pressure in atmospheres	
	Before treatment	After 2,4,5-T treatment
First 30 ml	8.5	8.3
Last 30 ml	8.2	8.8

that of the last sample increases above the initial level. It is possible that the increase in osmotic pressure represents not as much a loss of water as an entry of osmotically-active materials. Thus 2,4,5-T might either promote the inflow of solutes into the latex vessels along concentration gradients set up by the dilution after tapping or stimulate the release of solutes into the latex serum from the lutoids or other sites within the vessel itself. No definite conclusions on this point were possible from the present experiments.

Changes in Latex Total Solids Content in the Bark below the Tapping Cut

FERRAND (1941) described a technique for determining the total solids content of drop samples of latex obtained from small incisions in the bark. GOODING (1952a) modified the technique and used it to demonstrate the occurrence of dilution during flow in samples taken from within the 'drainage area' affected by a particular tapping. DE JONGE (1955) made further slight modifications in the method and demonstrated that the area of bark drained by flow and showing dilution increased after the 2,4,5-T treatment. De Jonge's technique was employed in the present work to obtain more information on changes in total solids content after tapping and the effects of an application of 2,4,5-T.

Dilution after tapping is demonstrated in *Table 3*. A sample was taken an inch below the cut immediately before tapping and another was collected from the latex on the surface of the cut as quickly as possible after reopening. Further samples were taken—two after 15 min when flow was proceeding only slowly and one after 30 min (below the tapping cut only) when flow had ceased. The volume and total solids content of the collected latex was also determined. Dilution was shown below the cut at 15 minutes but recovery was virtually complete after 30 minutes. The dilution recorded for the initial sample from the surface of the cut may be exaggerated by admixture with contents of cells other than latex vessels, since it displays a lower concentration than that of the bulk collected during the next 15 minutes. After 15 minutes, there is good agreement between all three measurements, the tapped latex showing the recovery in concentration expected at the end of flow.

Table 4 compares changes at three positions below the cut before and after 2,4,5-T application. Before tapping in the untreated tree there is a notably higher total solids content near the tapping cut (which was about 43 inches above the stock/scion union, the lowest sampling point being on the stock). The existence, similarly, of relatively large gradients in total solids contents in the neighbourhood of the tapping cut in the morning before tapping has been confirmed on several trees and can also be observed in some of the published data of GOODING (1952a) and DE JONGE (1955). It is not invariably seen, however, and occasional gradients in the opposite direction (i.e. with concentration increasing at greater distances below the cut) have been recorded. It is evident that the existence of such gradients in concentration within the latex vessels before the cut is reopened must modify the 'dilution effect' observed on the collected latex. Further, any change in these gradients following 2,4,5-T treatment will also change the pattern of 'dilution' observed during flow.

In the present case (*Table 4*), before treatment, flow had largely ceased by 30 minutes and partial recovery of the initial dilution had probably occurred at 5 inches below the cut. Some dilution is observed at all points, the 25-inch position showing the largest decline. After 2,4,5-T treatment, all the total solids contents are considerably reduced before reopening, but the effect is greatest at the 5-inch position, so that there are now only small differences in concentration between the three points, the highest concentration being on the

TABLE 3. CHANGES IN PERCENTAGE TOTAL SOLIDS CONTENT OF LATEX SAMPLES TAKEN ONE INCH BELOW THE TAPPING CUT, ON THE SURFACE OF THE CUT AND FROM THE COLLECTING VESSEL

(*a*) *Drop Latex Samples*

Sample	Time after tapping		
	0 minutes	15 minutes	30 minutes
One inch below cut	50.5*	47.6	50
Surface of cut	44.0†	47.5	—

* Taken immediately before tapping. † Taken immediately after tapping.

(*b*) *Collected Latex*

Measurement	0—15 minutes	15—30 minutes
Volume, ml	32	4
Total solids, %	45.4	47.9

TABLE 4. CHANGES IN PERCENTAGE TOTAL SOLIDS CONTENT OF LATEX AT VARYING DISTANCES BELOW THE TAPPING CUT

(Mean figures for five tappings before and eleven after 2,4,5-T treatment)

Period	Time relative to tapping (minutes)	Distance below tapping cut		
		5 inches	25 inches	50 inches
Before treatment*	− 5	57.1	51.5	52.7
	+ 30	54.5	44.1	50.3
	+120	53.1	51.0	53.0
After 2,4,5-T treatment†	− 5	43.3	42.8	44.8
	+ 30	32.3	35.2	41.9
	+120	36.1	35.5	42.0

* Mean latex yield 44 ml of 42.5% total solids. † Mean latex yield 116 ml of 39.9% total solids.

stock. Thirty minutes after tapping, when flow was still continuing, there was a marked gradient in concentration along the vessels with the 5-inch position showing the largest dilution effect. Some recovery in concentration had taken place two hours after tapping (when flow had almost ceased), particularly near the cut.

The difference in concentration between the drop sample taken five inches below the tapping cut immediately before reopening and the bulk latex collected was 14.6 percent on a total solids basis before the trees were treated, but only 3.4 percent after 2,4,5-T application. The total solids contents of successive latex samples before and after treatment appear to reflect the respective levels and gradients in total solids within the tree before tapping.

It is thought that such concentration gradients in osmotically inactive material (i.e. rubber hydrocarbon) are at least partly responsible for the discrepancies found between trends in osmotic pressure and total solids content of the collected latex. Clearly 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. More detailed work would be required in order to show whether osmotic pressure is a more reliable index of dilution, since the possibility of changes due to the entry or release of solutes, mentioned earlier, must be taken into account.

There is no strong evidence, from either the osmotic pressure or total solids records, of an exceptionally large inflow of water into the latex vessels during flow following 2,4,5-T treatment.

Changes in Total Solids Content of Latex in Rested Trees

It was thought possible that the large decrease in total solids content observed in latex samples taken near the cut before tapping following 2,4,5-T treatment (*Table 4*) might indicate a localised impairment of rubber biosynthesis in the neighbourhood of the treated band. To test this effect in the absence of the complications resulting from the increased losses of latex caused by 2,4,5-T treatment, two groups of trees were observed before and after resting from tapping, one group being treated at the start of the rest period. Total solids contents were measured on samples taken from six inches below the tapping cut. Observations covered a two-week period before and after resting and treatment. No clear differences in trends were observed between the two groups and, as shown in *Table 5*, both showed rather similar increases in latex total solids. Thus no effect on rubber biosynthesis *in vivo* could be demonstrated in the absence of tapping.

Repeated Reopening of the Tapping Cut and 2,4,5-T Treatment

It was evident that at some stage after tapping the cut ends of the latex vessels must become sealed since, at the next tapping, removal of a sliver of bark about a millimetre in thickness is necessary to re-open them. It seemed possible that the sealing process did,

TABLE 5. INCREASE OF TOTAL SOLIDS CONTENT OF LATEX IN TREES RESTED FROM TAPPING

(Samples taken six inches below the tapping cut on five occasions before and five after resting. Means for four trees per treatment)

Treatment	In tapping before treatment	Rested after treatment	Increase
Control, treated with carrier	48.4	57.5	9.1
2,4,5-T	46.5	55.1	8.6

in fact, play a major part in restricting latex flow. If so, it should be possible to increase yields by reopening the cut at frequent intervals, while flow was still continuing. A group of Tjir 1 trees with a mean period of flow after tapping of less than thirty minutes was selected. After a first normal tapping in which yields, rates of flow and total solids contents were measured, two trees were allocated to each of the following treatments:

I — normal tapping (S/2.d/2.100%)

II — normal tapping with 2,4,5-T treatment after the third tapping day.

III — tapping cut reopened at 15, 30, 45, 60 and 75 minutes after the initial tapping.

IV — as III, with 2,4,5-T treatment after the third tapping day.

As will be seen in *Figure 6A*, the control trees (I) showed a tendency to increase in yield during the month of observation (which is normal during March–April). On the second and third tapping days (before 2,4,5-T treatment) the repeated reopening treatments both showed almost a three-fold increase in latex yield. Application of 2,4,5-T brought about similar large increases in both the single-tapped and repeated-tapped trees. The yield of the reopened 2,4,5-T treatment (IV) reached about five times that of the control (I).

The total solids contents (*Figure 6B*), as might be anticipated, were markedly lower in latex from trees treated with 2,4,5-T than in latex from trees receiving the same tapping treatment without the growth regulator. The yields from repeated reopening alone (III) were substantially similar to those obtained by a single tapping after 2,4,5-T treatment (II), but the effect on total solids content was notably smaller. Indeed, it was not until the third day of repeated tapping that the solids content fell below that of the control. Similarly it was not until the seventh tapping that the total solids content of IV fell below that of II. This suggests that, despite the lack of positive evidence in the earlier experiments, 2,4,5-T does have an effect in lowering the rubber content of the collected latex which

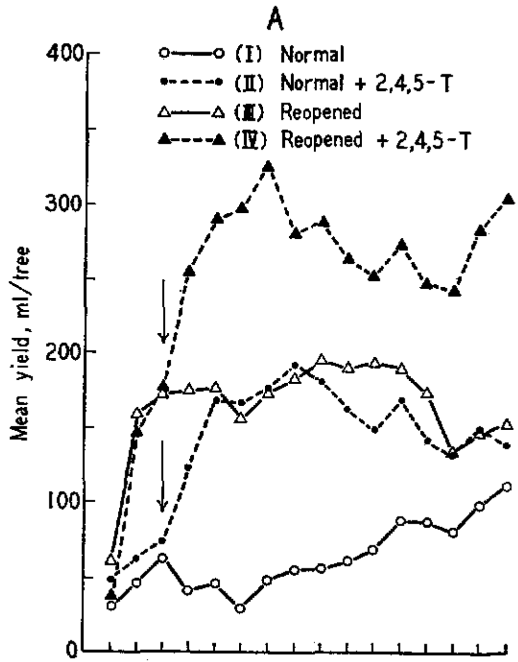


Figure 6. Effect of 2,4,5-T treatment and reopening the tapping cut at fifteen-minute intervals on volume of latex yielded (A) and its total solids content (B).

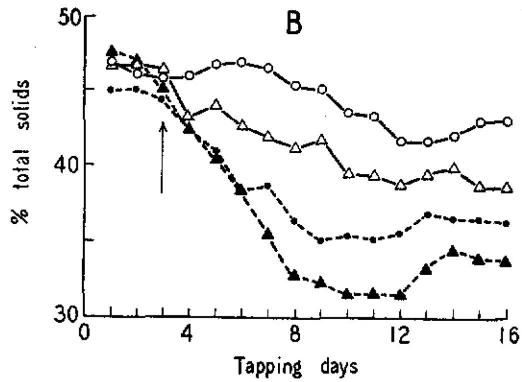
○ — ○ *Normal S/2.d/2.100% tapping.*

● - - ● *Normal tapping, treated with 2,4,5-T.*

△ — △ *Reopened at 15, 30, 45, 60 and 75 minutes after the initial tapping.*

▲ - - ▲ *Reopened at 15, 30, 45, 60 and 70 minutes after the initial tapping, treated with 2,4,5-T.*

All trees tapped normally on first day. Repeated reopening commenced on second tapping day and 2,4,5-T was applied, as indicated by the arrows, after the third tapping day.



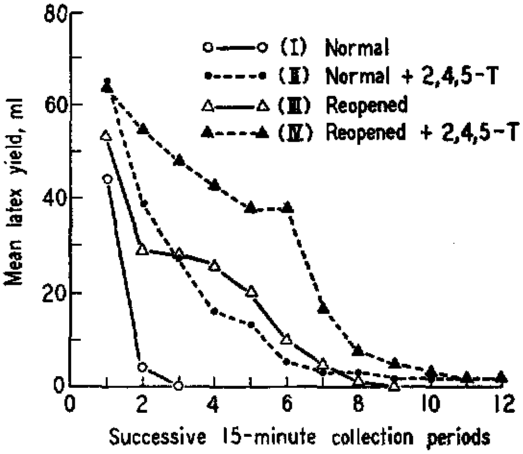


Figure 7. Comparison of effects of 2,4,5-T treatment and repeated reopening of the tapping cut on rates of latex flow during successive fifteen-minute intervals after the initial tapping. Data from the seventh tapping day in the experiment shown in Figure 6.

○ — ○ *Normal S/2.d/2.100% tapping.*
● - - ● *Normal tapping, treated with 2,4,5-T.*
△ — △ *Reopened at 15, 30, 45, 60 and 75 minutes after the initial tapping.*
▲ - - ▲ *Reopened at 15, 30, 45, 60 and 75 minutes after the initial tapping, treated with 2,4,5-T.*

cannot be explained entirely in terms of the greater losses at previous tappings.

To compare the effects of 2,4,5-T and repeated reopening more closely, latex yields for successive 15-minute periods on the seventh tapping day are given (*Figure 7*). Flow in the control (I) decreased rapidly, ceasing entirely within 30 minutes, so that no yield was obtained in the third period. Treatment with 2,4,5-T *gave a higher yield in the first period and the* rate fell less rapidly, flow continuing slowly until the end of observations. Repeated tapping alone (III) had a lower yield in the first 15 minutes than following 2,4,5-T application, but re-opening the cut greatly slowed down the decline in yields in the successive periods. Flow stopped fairly rapidly after the last tapping. Re-opening and treatment with 2,4,5-T (IV) produced an increased yield during each period in much the same way as it had after *a single tapping.*

It seemed certain, from the effect of re-opening the cut, that flow was severely restricted in these trees by some process occurring within a millimetre of the cut surface. It was equally clear that 2,4,5-T must have delayed this process, directly or indirectly, since it greatly prolonged flow after a single tapping. To investigate this point further, rates of flow were measured at half-minute intervals on two similar trees (Nos. 37 and 40). On every second tapping day the cuts on both trees were re-opened, first ten minutes after the initial tapping, and then again, ten minutes later. On the alternate tapping days a normal, single tapping was given. After the first cycle of normal and repeated tapping, tree No. 40 was treated with 2,4,5-T in the same way as in the

TABLE 6. LATEX VOLUMES AND TOTAL SOLIDS CONTENTS FOLLOWING REPEATED RE-OPENING AND 2,4,5-T TREATMENT

Period	Date	No. of times cut opened	Tree No. 37—Control		Tree No. 40—2,4,5-T	
			Vol. ml	% T.S. range	Vol. ml	% T.S. range
Before 2,4,5-T treatment	18/3	1	20	52.4–51.1	22	50.9–50.6
	20/3	3	34	51.3–50.0	31	51.0–50.1
After 2,4,5-T treatment	22/3	1	18	51.8–50.7	71	49.2–47.4
	24/3	3	34	52.8–49.5	78	51.0–45.5

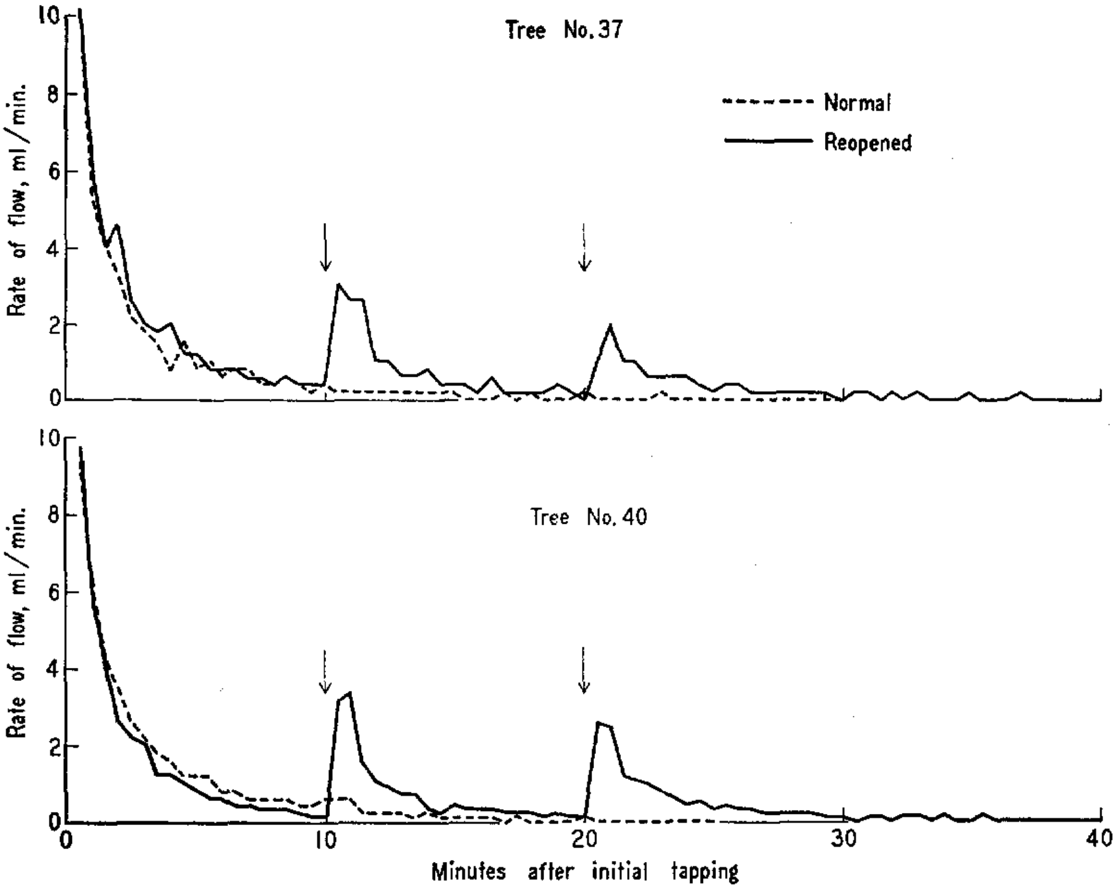


Figure 8. Rate of latex flow recorded at half-minute intervals after tapping for two Tjir 1 trees. Comparison of normal S/2.d/2.100% tapping (broken line) with re-opening the cut, as indicated by arrows, 10 and 20 minutes after the initial tapping (solid line).

previous experiment. Tree No. 37 served as a control. The resultant yields are given in *Table 6*.

It will be seen that, in the absence of 2,4,5-T treatment, the reopening treatment (which was much less intensive than in the previous experiment) causes a substantial increase in the volume of latex obtained in both trees. The 2,4,5-T application led to a large increase in yield and repeated reopening than had relatively little effect. The latex total solids content was recorded at intervals during flow and the ranges encountered are listed (*Table 6*). The repeated reopening treatment led to a slightly lower solids content, but a much more marked fall was observed in the 2,4,5-T treatment.

Figure 8 gives the rates of flow for the first cycle of normal and repeated tapping (before 2,4,5-T treatment). During the normal tapping the rate of flow fell sharply to a low value within ten minutes and flow had largely ceased by 20 minutes. Reopening the cut after ten minutes caused a sharp increase in flow rate which then declined again to a low level within another ten minutes. The second reopening led to a similar sequence of events, the size of the peak being reduced compared

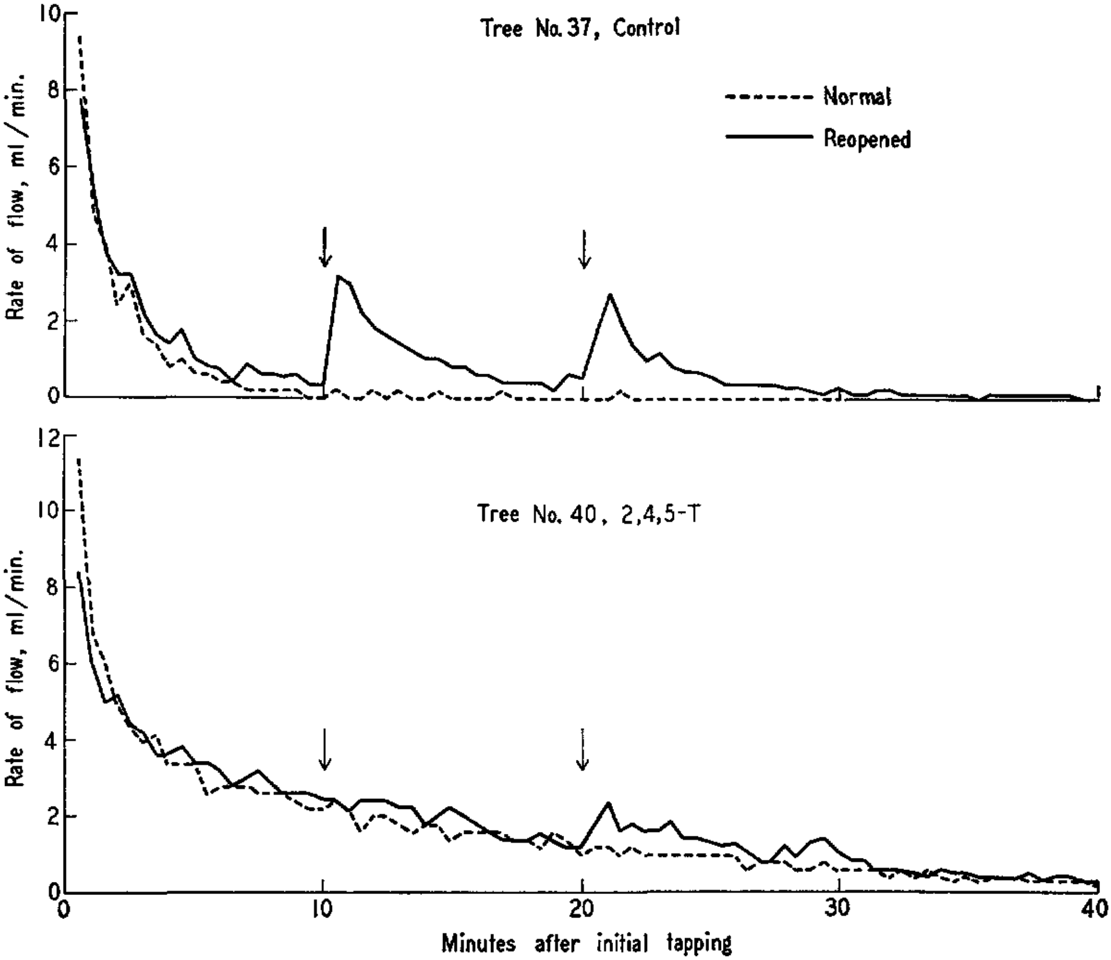


Figure 9. Rate of latex flow recorded at half-minute intervals after tapping for two Tjir 1 trees, one control (No. 37) and one treated with 2,4,5-T (No. 40). Comparison between normal S/2.d/2.100% tapping (broken line) with re-opening the cut, as indicated by arrows, 10 and 20 minutes after the initial tapping (solid line).

with the previous one. These results fully confirm those of the first experiment.

The flow rates for the second sequence of normal and repeated tapping, after 2,4,5-T treatment of tree No. 40, are depicted in *Figure 9*. The results for the control, No. 37, are very similar to those for the first cycle. Normal tapping of tree No. 40, gave the expected 2,4,5-T effect, with a much slower fall-off in the rate of flow, which was still at a high level at ten minutes, and continued to fall only slowly thereafter. Under these conditions, re-opening the cut at ten minutes after the initial tapping had no apparent effect on the flow-rate. Even after 20 minutes, the effect was only small.

These observations indicate that, in the tree treated with 2,4,5-T no significant obstruction to flow appeared within the last millimetre of tissue during the first ten minutes and even

TABLE 7. RATE OF LATEX FLOW IMMEDIATELY FOLLOWING REOPENING OF THE TAPPING CUT BEFORE AND AFTER 2,4,5-T TREATMENT

(Comparison of measurements on 20/3 and 24/3, before and after 2,4,5-T treatment)

Time interval after tapping (minutes)	Rate of flow in ml per minute					
	Initial tapping		Re-opened after 10 min.		Re-opened after 20 min.	
	20/3	24/3	20/3	24/3	20/3	24/3
0.0–0.5	9.8	8.4	3.2	2.4	2.6	1.8
0.5–1.0	5.6	6.0	3.4	2.2	2.5	2.4
1.0–1.5	4.2	5.0	1.6	2.4	1.2	1.6
1.5–2.0	2.6	5.2	1.0	2.4	1.1	1.8
2.0–2.5	2.2	4.4	0.9	2.4	1.0	1.6

after twenty minutes, the restriction of flow due to this cause was only slight. In contrast, in the untreated trees, flow was evidently severely restricted by such an obstruction well within the ten-minute period. The rapidity of formation of the barrier may be observed from *Table 7*, which compares the rates of flow occurring shortly after each of the three tappings of tree No. 40 on repeated re-opening tapping days, before and after 2,4,5-T application. In each case the initial rate of flow was slightly lower following 2,4,5-T treatment, but the position was reversed within 1.5 minutes. Thus it appears that, before treatment, latex flow became impeded within 1.5 minutes of tapping.

DISCUSSION

Among the listed mechanisms by which growth regulators might promote latex flow, it now appears that at least two need not be considered further. The dendrometer measurements, although not entirely satisfactory, are clearly against any mechanism involving changes in latex vessel collapse. Changes in latex viscosity were largely uncorrelated with yield trends and, although there are obviously dangers in extrapolating such measurements on collected latex to conditions within the latex vessels, it does seem rather unlikely that the major effect of 2,4,5-T treatment is to be found here.

On the other hand, the repeated reopening experiments showed clearly that flow may be severely restricted within a very short time after tapping, by some process occurring at, or within a millimetre of, the cut ends of the latex vessels. Flow was greatly prolonged as a result of 2,4,5-T treatment and, under the conditions of the second experiment, reopening the cut ten minutes after the initial tapping lost its effectiveness following the 2,4,5-T application. These results indicate that 2,4,5-T must, directly or indirectly, delay the flow-restricting process.

The largely additive effect of combining the 2,4,5-T and reopening treatments in the first experiment might be thought to be evidence that 2,4,5-T has some other promotive effect, besides delaying latex vessel closure. However, this may well be an example of the 'additive' effects frequently observed when two similar sub-optimal treatments are combined. This would imply that neither treatment alone was capable of preventing some restriction of flow within the fifteen-minute period between successive reopenings.

If a second promotive effect of 2,4,5-T action is involved, then either an increase in initial osmotic and turgor pressures or a change in membrane permeability which might permit turgor to be maintained better following the initial dilution, must be considered. The two contrasting flow curves for different times after 2,4,5-T treatment (*Figure 4B*), certainly suggest the possibility that, soon after treatment, the major effect is on initial flow rate, whereas later the yield increase is due to a prolonged period of flow. It is however possible that, in view of the relatively large initial sample (30 ml), the observed rate of flow was, in fact, largely governed by the same flow-restricting process. Differences in the effect of 2,4,5-T at the early and late stages of response might be interpreted in terms of its translocation within the latex drainage area. Thus, if the effect is on the latex itself, it will be mainly observed on the latex nearest to the cut (and the point of 2,4,5-T application) in the first few days after application. This latex will flow out of the cut first, giving improved yields, and then be replaced by latex from more distant areas which is more or less unaffected, explaining the relatively small effects observed at the end of flow. Later, when the 2,4,5-T has become more widely distributed, greatly prolonged flow results. Whether an increased rate of flow occurs in the initial samples after tapping under these conditions might depend on the extent to which osmotic and turgor pressures had been reduced by the excessive removal of metabolites for latex regeneration.

There is certainly no direct evidence from the freezing-point determinations of any increase in initial osmotic pressure, but the present technique, involving the use of relatively large samples of latex with an unknown dilution error, is clearly not entirely satisfactory for this purpose. Work is proceeding on more refined methods.

Neither the freezing-point method nor the study of changes in total solids content has proved entirely satisfactory as a measure of the extent of dilution by water from surrounding cells. Some indications of a relatively rapid entry or release of osmotically-active material were obtained, casting doubts on the validity of osmotic pressure measurements, particularly on the latex collected at the cut, as a measure of water entry. Rubber contents should be free from this error but, unfortunately, the gradients in concentration observed near the tapping cut make interpretation of such measurements almost equally difficult. Nevertheless, it must be said that there is no good evidence at present for suggesting that changes in the 'dilution reaction' play any large part in the 2,4,5-T effect.

There seem to be definite indications that 2,4,5-T may have an effect on the concentration of rubber hydrocarbon within the latex vessels and in the collected latex which cannot be explained purely in terms of the additional dilution resulting from increased yields. But no effect could be detected in rested trees. Thus, if there is an inhibitory effect on rubber bio-synthesis, it is only manifest in regularly-tapped trees, suggesting an indirect action involving changes occurring within the latex vessel during flow.

The nature of the process which restricts flow so quickly after tapping and the manner in which 2,4,5-T delays its operation is obviously of prime interest and importance. TAYSUM (1961a) reported the establishment of a bacterial population in the cut ends of the latex vessel following regular tapping and showed (1961b) that latex yields may be increased by application of antibiotics. It seems however, that growth regulators tend to cause an increase rather than a reduction in the bacterial population in the tapped latex (NGUYEN HUNG CHAT, PUJARNISCLE AND D'AUZAC, 1961) and TAYSUM (1961b) himself suggests that 2,4-D and 2,4,5-T tend to worsen the bacterial position in the tree. In the present work, the considerable additional excision of tissue under the cut in the first repeated tapping treatment might have been expected to reduce bacterial contamination within the latex vessels, but flow still declined rather rapidly after the final re-opening. Indeed, the rapidity with which the flow-restricting process occurred, tends to argue against an effect involving the establishment of a population of micro-organisms. The apparent absence of any localised barrier to flow, ten minutes after tapping following 2,4,5-T treatment in the second experiment, contrasts with the relatively high bacterial counts noted above for similar trees. Thus it seems rather unlikely that 2,4,5-T, in prolonging flow, is acting directly or indirectly on a bacterial mechanism.

If it is assumed that the end-product of the process which restricts flow is a 'plug' of coagulated rubber at or near the end of the latex vessel, then it is not inconceivable that the lower rubber content of latex in trees treated with 2,4,5-T might itself cause some delay in building up a coherent seal. It is interesting to note that the lower rubber content resulting from repeated tapping (without 2,4,5-T treatment) is accompanied by an increased yield during the first fifteen minutes and a somewhat prolonged flow after the final tapping. These effects are more marked in the 2,4,5-T treatments, with their still lower rubber contents.

A marked tendency has been noted for samples of latex collected immediately after tapping to show rather rapid partial pre-coagulation, particularly in trees treated with 2,4,5-T. This obviously runs counter to any possible effect of 2,4,5-T in improving the 'stability' of latex, as usually measured in the laboratory. Again caution is required in the interpretation of such results on latex collected after tapping, since it may undergo changes during flow and after leaving the tree. This phenomenon is still under investigation.

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