# ON THE FORMATION OF NEW RUBBER IN THE LATEX-VESSELS OF HEVEA

by

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With 2 textfigures.

## § 1. Introduction and method of research.

In various plant-families there occurs a system of laticiferous elements the significance of which is still wrapped in the dark. The road to get an insight into this problem leads through investigation into the function of the system of laticiferous vessels. Hevea has appeared to be particularly suitable for these investigations on account of the property of the latexvessels to yield a certain quantity of latex on being wounded, without its resulting in detriment or irreversible change in the normal processes. Divers of such specialised systems as the system of laticiferous vessels occur in the plant. Leaving the system of water-conducting vessels out of account, we find in the bast a.o. a system of continuous sieve-vessels, one of elements containing tannin and one of parenchymatous elements containing a.o. carbohydrates. A further investigation into these different systems of primary importance for metabolism, is hardly practicable. In the system of latex-vessels of Hevea however we are in more favorable circumstances, because we are able to subject the composition of the latex in definite parts of the system to an accurate quantitative examination and by doing so to carry out an investigation into the metabolism of the latex.

The latex of Hevea contains as principal constituents:

rubber, resin, nitrogenous substances, quebrachite and salts. Several of these substances have already been examined quantitatively. On the rubber-formation we wish to give some quantitative data in this communication.

Latex from old Hevea trees consists for about 55 % of rubber. Such latex is very viscous and on our making an opening in the latex-vessels it flows slowly and in slight quantity. On flowing out the latex alters its composition, which alteration is chiefly due to a dilution with water. Cutting the latex-vessels puts an end to the turgor and water filters from the adjacent cells into the latex-vessels (4)[1]. This change in the latex during the flow should always be taken into consideration on taking samples. It has however appeared that this dilution is less inconvenient, when the latex is less viscous in consequence of a lower rubberconcentration and when we restrict ourselves to taking samples only from the first flow of latex. In this way it is possible to get a sufficiently reliable sample of the latex present in the system of laticiferous vessels before the cutting. The sample to be examined must hold at least some cc latex, so that, therefore, the latex must fill an equal volume in the tree before the flowing. If therefore for the examination a tree is chosen of which the system of laticiferous vessels is vigorously developed, the latex examined will be derived from a comparatively small bast-area. On comparing latices of trees of differently developed systems of latex-vessels we shall have to take into account that the area yielding an equal quantity of latex will be larger in trees with few laticiferous vessels in the bast, than in trees with a strongly developed latex-vessel-system.

For determining the rubbercontent of the latex the nitrosite-method might be used. As however according to Bobilioff (6) this method is neither simple in prac-

[1]) The figures in brackets relate to the literature cited at the close of this publication.

tice nor offering great accuracy, it has not been used in these researches. When latex is evaporated, the quantity of dry substance consists for the greater part of rubber, for a small part of other substances occurring in the latex, the composition of which however does not vary considerably. In the determination of the dry residue of the latex we have therefore already a useful standard of the rubber-content for many purposes. When, as is the object of these researches, we want to know the increase in rubber-content, in which therefore the difference of two determinations of dry residue is used, but a slight error will be made by taking the dry substance-content instead of the rubber-content. Even better than the determination of the dry residue of the latex a determination can be made of the dry residue of the substances precipitated from the latex on coagulation. For this purpose the latex should be diluted with much water, e.g. a tenfold quantity and coagulated with weak acetic acid. Besides the rubber with the resin but few other substances precipitate, causing no error worth mentioning in the rubber-content. The errors due to changes in the latex during the flow are considerably greater than those resulting from the determination of the dry residue of latex or coagulated rubber.

## § 2. Review of former researches on formation of new latex.

In order to trace whether in the laticiferous vessels there has occurred formation of new rubber, various methods may be followed. For Hevea De Jong and Bobilioff (5) isolated a piece of the bast, so that the laticiferous vessels of the isolated piece were in no way connected with the other vessels of the tree. By tracing how much rubber was initially contained by this piece of bast and how much rubber could be tapped in totality by daily cutting the latex-vessels for some months together, it could be demonstrated that from this isolated piece of bast there flowed more rubber than was initially present so that new rubber must have been formed. Bobilioff moreover showed that the rubber-formation was not due to addition of new laticiferous vessels to those already present and that therefore a secondary latex-formation has occurred in the old latex-vessels.

Some quantitative determinations of the increase in concentration we already communicated before (2). In a Hevea-tree, daily cut and daily yielding latex, the content of dry substance of the latex had diminished to 39.8 %. After a 19 days' rest the latex-vessels were opened in the same place. By that time the flowing latex contained 43.1 % dry residue. On our taking a new sample after a fortnight it had risen to 48.8 %, in the 16 days then following to 51.8 % and next in 27 days to 56.3 %. 91 days after the first sample was taken the content had increased to 57.3 %, i.e. a total rise of 17.5 % in 90 days, that is about 0.2 % per day.

In Fig. 1 a graphic representation has been given of the increase in dry substance in four trees.

The course of the curves is fairly analogous. Trees 1 and 2 have an equal degree of increase of concentration. The concentration of the latex of these trees increases from 0.1 to 0.5 % per day. Trees 25 and 49 show a slightly slower increase of rubber-content. The first days after stopping the tapping somewhat lower figures have been found. This might give the impression that the increase of rubber-content does not attain its full strength until some days have passed, but from such slight differences no conclusions should be drawn, because the withdrawing of latex and the sample-taking procure slight deviations in the concentration of the latex. We also refer to the course of the curve on the increase in concentration in tree 1 in 1922 in connection with which we pointed out (l.c.p. 8) the possibility that for this experimental tree the quicker

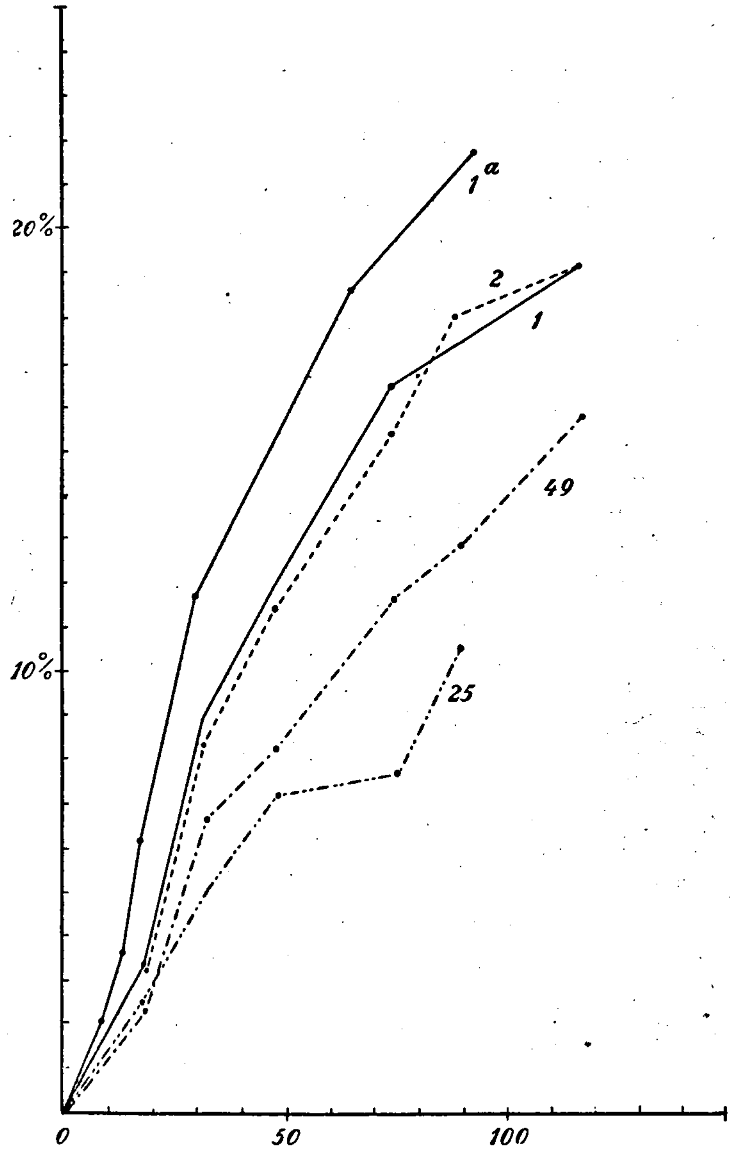


Fig. 1. Increase of latexconcentration after stopping of tapping. On the abscissa the number of days after stopping, on the ordinate the increase in concentration of the latex The curves 1 2 25 and 49 contain the figures for tree 1 2 25 and 49 in 1921. Curve 1a for tree 1 in 1922.

increase of concentration in 1922 as compared to 1921 might be due to the preceding period of one year's rest. The experiments mentioned below show, that this supposition is so far correct, that the general nutritional condition of the tree may have a considerable influence on the degree of the increase of the rubber-content. We already pointed out then, that determining the rate of this process during the first days is not so simple an affair. Each tapping causes a loss of latex and this loss must be made good before there can be question of a new increase of concentration. Therefore the smallest possible quantity of latex should be withdrawn for taking samples. Artificial arrest of the flow of the latex which would be superfluous for the determination of the dry residue has proved hardly practicable. Nor is making a small aperture for which few latex-vessels are opened, desirable, because collecting a sufficient quantity of the latex flowing in the first minutes is important, as afterwards the composition may have altered too much. In this way it seems impossible for us to get more accurate figures for the rubber-concentration in the first days.

The final concentration at which the process of increase of rubberconcentration is brought to a standstill, varies individually. In tree 49 there was never found a higher value for totally dry substance than 55.5 %, in tree 1 the highest concentration amounted to 62.9 %, in tree 25 to 60.4 %, in tree 2 to 61.6 %. To this we shall revert when discussing the next experimental series.

## § 3. Experiments on the increase of rubber-concentration.

The phenomenon of the concentration-increase of rubber discussed above has been examined in a great number of plants. For these experiments it seemed in the first place desirable to have a great number of observations at our disposal in order to get a better insight into the fact whether the phenomena stated for some trees hold good for all and whether the process of the concentration-increase always proceeds identically. In these experiments the latex was coagulated, by which we procured somewhat purer values for the rubber-content of the latex. The objections in the preceding series due to changes in the latex during the flow also obtain in this case, partly they are even stronger, because we also experimented with plants yielding a less great quantity of latex, which as we already saw, aggravated that error. Great accuracy however was not necessary in these experiments, because the period between two observations was much longer in this case, always a month, so that the concentration-differences were much greater. Yet some values in which the concentration of the rubber was very high and the concentration-increase slight or nought proved, that the imperfections in the sample-taking act a part, though it be slight, because in some cases an apparent decrease in concentration of the latex was found. It is not impossible that decrease in concentration of the latex really takes place in the laticiferous vessels, on account of these vessels growing more turgescent, but we have the impression that this cannot be the question in these experiments. Of a decrease in the quantity of rubber in the latex in consequence of disappearance of rubber in the metabolism no indication has hitherto been found. These slight deviations are not of any influence on the interpretation of our experiments.

The experiments were made on a group of trees in the experimental gardens of Kaliwining near Djember in Eastern Java. Before our starting the experiment the trees had been tapped every other day, so that the rubber-content of the latex was considerably lower than the concentration in untapped trees. During the first three months, February, March and April, the experimental group A was given a rest and the group B was tapped daily. After this first period of three months group A was daily tapped during three months, May, June and July and B was allowed a rest. The three following months, August, September and October group A was resting and B tapped and the last three months, November, December and January group A tapped and B resting. By a monthly determination of the rubber-concentration of the trees which were not being tapped, data were obtained how the concentration of the rubber rises in the various months of the year, in which groups of four consecutive determinations refer to a same group of trees. From the observations made on 200 trees those of 40 trees of group A and 39 trees of group B are joined in a table. (Table 3 at the close of this publication). As to the other trees it was not possible to make a complete series of observations, because in one or more months no sufficient quantity of latex flowed on cutting the trees to enable us to determine the rubber-concentration. In the table there has likewise been recorded the increase of rubber-concentration in the month passed between two observations. From the observations made on the individual trees the averages have been computed for the increase of the rubber-content in the successive months. The increase of rubber-content is expressed in percentage of the latex. In the table subjoined the average increase of the rubber-content per month has been recorded.

TABLE 1.

| | | | | | | |
|---|---|---|---|---|---|---|
| | Febr. | March | April | Aug. | Sept. | Oct. |
| group A ..... | 7.4 | 1.9 | 3.9 | 4.3 | 8.1 | 4.1 |
| | May | June | July | Nov. | Dec. | Jan. |
| group B ..... | 8.7 | 4.9 | 2.2 | 6.8 | 5.2 | 1.0 |

Average monthly increase of the rubber-concentration in three-monthly periods of rest.

In every month therefore an increase of the rubber-content has occurred, the average strength of which varies from 8.7 to 1 %.

In general the increase of the rubber-content has been strongest during the first month of the three-monthly period of rest and decreases during the second and third months. An exception to this is the period August, September, October, where during the first month group A has increased its rubber-content less than during the second month. Nor do we find a gradual decrease in the period February, March, April, because in the second month there is formed less than in the third. From these figures on the average increase of the rubber-content, we may conclude for the present, that in general in the first month of the period of rest the rubber-concentration increases most, next diminishes in the second and third months, but exceptions to this occur, on account of which now the second month shows a stronger increase than the first, now the second gives smaller values than the third month.

In order to get an insight into the cause of this phenomenon, it is necessary to study the figures obtained for the various trees more closely. Let us first consider the phenomenon of the increase of concentration for some trees individually. In the physiological research on Hevea it has always appeared, that there are not two trees behaving in exactly the same way. On this subject numerous data have been recorded, especially on the phenomena concerning wintering. In this case too we see, that the trees individually behave much more deviating than we might conclude from the average figures. As an example we choose tree 168, a tree yielding a great deal of latex, the observations of which are sure to be reliable. At the beginning of the period of rest this tree had a rubber-concentration of 31 %, after 1 month it had increased to 41.6 %, i.e. an increase of 10.6 % or 0.35 % per day. This figure corresponds more or less with the value found for other good yielders mentioned in § 2. In the second month this tree shows an increase of 5.3 %, in the third month of 5.1 %, by that time the concentration has increased to 52 %. Next this tree is tapped daily for 3 months, which makes the concentration fall to 40.6 %. Then a new period of rest sets in. In the first month of rest the concentration does not increase as in the first period with 10.6 %, but only with 1.9 %. In the second month however from 42.5 % to 52.8 % that is 10.3 %. In the third month the increase is 5.7 %. From these observations some important conclusions may be drawn.

Till now it has been the general opinion in the literature on rubber-tapping, that the so called *wound-stimulus* has an important influence as well on the flow of latex as on the formation of new substances in the latex-vessels. In consequence of this view of wound-stimulus too much stress was laid on the supposition, that abnormal processes are taking place after opening the latex-vessels. The data given here make it clear, that in the increase of rubber-concentration[1]) the wound-stimulus does not play a part and that the process of increase of rubber-concentration is a normal physiological response caused by different factors which will be analysed here after. On our reverting to our figures got from tree 168, it appears to be possible, that during the first month a low rubber-concentration is maintained, from which it follows, that in those circumstances one of the conditions necessary for the fulfilment of the increase of rubber-concentration was not satisfied. As the wound stimulus must be always the same at the moment of wounding, we may conclude that anyhow the process must also depend on other factors. By its side it appears that in the second month of the second period of rest the rubber-

[1]) In a subsequent publication, which will appear in this periodical I will demonstrate, that also the latex-flow after wounding has nothing to do with a wound-stimulus.

concentration can increase as much as in the first month of the first period. Therefore we must accept, that not a stimulus brought about by the wounding and which afterwards grows weaker and weaker produces the increase of concentration of the rubber, for that would make a lack of increase in the first month and a high increase in the second incomprehensible. *The increase of concentration of the rubber, therefore, is not caused by the wound-stimulus on cutting the laticiferous vessels,* but by conditions we shall now further analyse. In 30 of the 40 trees examined in August we find the same phenomenon that in August, the first month of the period of rest, the rubber-content increases less than in September, the second month. This is sufficient proof, that we have to deal here with a general phenomenon.

From the figures given in table 3 for the individual trees, we will mention here some more. The highest values for the increase of the rubber-content during a month we find in tree 46 in the first month of the period of rest, where it amounts to 17 %, while we find a value of 17.6 % for tree 70 in the second month of the period of rest. The increase of rubber therefore may attain equally high values during the second month as during the first. In the third month of a period of rest such high values have never been stated. Here the highest values are found for tree 158, where the increase amounts to 9.7 % in April, for tree 159 to 9.5 % in April, for tree 15 to 8.6 % in October, and for tree 400 to 8.1 % in July. From the nature of things the average concentration of the trees will be greater in the third month of rest than in the preceding months. It does not seem impossible that there exists some relation between the strength of the increase of rubber-concentration and the quantity of rubber already present in the latex. This could explain the lower increase of the rubber-concentration in the third month of the period of rest. We will therefore now examine our figures to discover wether it is possible to say something about the relation between the rubberconcentration already present and the increase of it during the next month.

The above observations on tree 168 indicate that with

TABLE II.

| | Rubber-concentration at the beginning of each month. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20–25% | 25–30% | 30–35% | 35–40% | 40–45% | 45–50% | 50–55% | 55–60% |
| February ... | 9.1 (1) | 8.4 (7) | 8.0 (16) | 6.6 (12) | 5.9 ( 4) | — | — | — |
| March ..... | — | — | 3.5 ( 4) | 2.5 (11) | 2.1 (16) | 0.7 ( 8) | 0.0 ( 1) | — |
| April ...... | — | — | — | 5.0 ( 9) | 4.3 (18) | 2.6 (13) | — | — |
| May ....... | 10.4 (4) | 9.8 (9) | 8.3 (16) | 7.5 (10) | — | — | — | — |
| June ....... | — | 3.9 (2) | 5.3 ( 2) | 5.3 (12) | 4.9 (16) | 4.1 ( 7) | — | — |
| July ....... | — | — | 3.0 ( 2) | 3.1 ( 4) | 3.0 (10) | 1.5 (18) | 2.2 ( 5) | — |
| Aug. ....... | — | 4.1 (5) | 4.7 (16) | 5.2 (11) | 2.6 ( 8) | — | — | — |
| Sept. ...... | — | 13.0 (1) | 12.1 (8) | 7.6 (12) | 6.7 (13) | 6.0 ( 6) | — | — |
| Oct. ....... | — | — | — | 5.9 ( 1) | 5.3 (11) | 4.2 (16) | 2.7 (12) | — |
| Nov. ....... | — | 8.9 (2) | 8.0 (12) | 6.9 (14) | 4.9 (10) | 4.7 ( 1) | — | — |
| Dec. ....... | — | — | 8.9 ( 1) | 6.1 ( 7) | 6.0 (16) | 4.0 (14) | 3.7 ( 1) | — |
| January .... | — | — | — | — | 0.9 ( 5) | 1.2 (19) | 0.8 (13) | 0.0 (1) |

Increase of rubber-concentration in different months arranged over eight classes according to the rubber-concentration at the beginning of each month. Each value is an average of all the trees of which the rubber-concentration of the latex belongs to a certain class. The number of trees is put into brackets.

an equal rubber-content the increase of concentration in the same tree may be different. During the second month of the first period of rest we found an increase of 5.3 % with an initial rubber-content of 41.6 % and during the second month of the second period of rest with an initial content of 42.5 % an increase of 10.3 %. This however only proves, that other factors may exercise a greater influence than the rubber-concentration. In order to trace whether there is a relation between rubber-content and rate of increase of rubber-concentration all observations of table 3 have been arranged in a number of classes with increasing rubber-content, 20—25 %, 25—30 %, etc. Next it has been computed for every month how strong the average increase of the rubber-content is for the plants, belonging to each of these classes. Of the 40 plants examined in February (c.f. table II) one had an initial concentration falling in class 20—25, 7 plants belonged in class 25—30, 16 in class 30—35, 12 in class 35—40, 4 in class 40—45. As an average concentration-increase in each of these classes we find respectively 9.1 %, 8.4 %, 8.0 %, 6.6 % and 5.9 %, i.e. an obvious connection between initial concentration and strength of rubber-increase. For the following months it has been computed in the same way. In table 2 the averages obtained have been recorded.

Though the values obtained in the different months vary greatly we see in every month, that there is a connection between initial concentration and strength of the rubber-increase. So we may conclude, that *the strength of the increase of rubber-concentration depends on the rubber-concentration present,*the lower it is, the stronger the increase will be. This may also be expressed as follows: the increase of rubber-concentration is the stronger according as the quantity of serum in the latex is greater.

On the ground of this conclusion in order to compare the strengths of the increase of concentration in the successive months, we shall have to start with equal initial concentrations. The average figures of table 1 must not be used for this purpose, since they do not refer to an equal average initial concentration. The arrangement in classes however of the results of our experiments in table 2 renders the comparison possible to us. Best suited for this purpose are the classes 35—40 % and 40—45 %, occurring in nearly all months. In Fig. 2 these figures have been graphically represented for the classes 35—40 and 40—45. The deficient values have been estimated by extrapolation.

As shown in the figure the course of the curves is fairly identical in the two classes. In March and January the

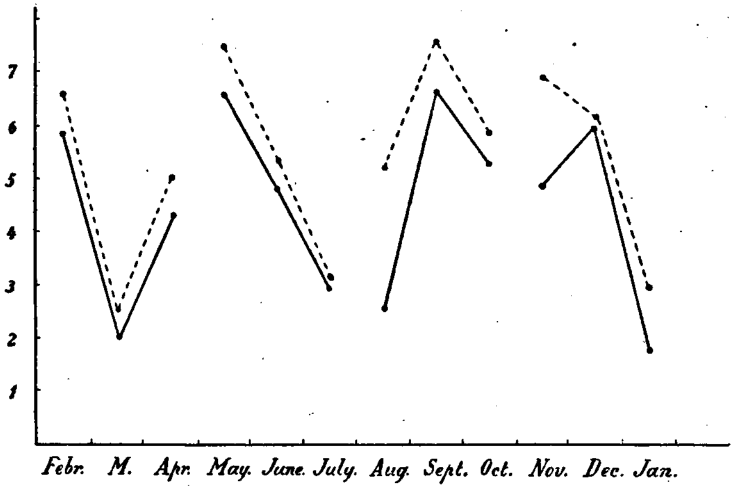


Fig. 2. Graphic representation of the values from table II for the increase of rubber-concentration from the classes with an initial rubbercontent of 35—40 % (dotted lines) and of 40—45 % (solid lines).

increase of concentration is slight, next July, August, April, June and October follow. The increase of concentration is strong in September, May, February, November and December. The difference in strength of the increase of rubber-concentration in these months may be due to various causes. In the first place each experiment is continued for three months. A certain influence of the periodicity of the experimentation we find in the strong increase in every first month of rest: February, May and November, only August making an exception. But by the side of this periodicity which is a result of the experimental period of three months we are certain of an other influence. On the nature of that influence we can derive but little from our experiments. It is a striking fact that in the months of July and August the increase of concentration is abnormally slight, on the other hand in September and October great. This cannot be due to external conditions such as the dry monsoon, as in 1925 the rains did only set in after these months. The cause will rather have to be found in internal factors. We think of internal changes in the tree in connection with wintering which falls for these trees in the very months of July and August. In former researches (1 and 2) we found the period of wintering to be of great importance for the metabolism of Hevea, which is among other things strongly evident from the periodicity of the flow of latex. We may suppose that in some way this process of increase of the rubber-concentration is influenced by the metabolism which is entirely altered by the wintering. We will revert to this in § 5.

## § 4. Survey of experimental results.

From the researches above-mentioned we may conclude as follows:

1. when by withdrawing latex the rubber-concentration is artificially decreased in the latex-vessels, an increase of rubber-concentration takes place.
2. the strength of this process is greater according as the latex has a lower rubber-content;
3. the strength is determined by factors closely related to the physiological condition in which the tree is;
4. the increase of concentration of the rubber is not due to the wound-stimulus on opening the latex-vessels.

## § 5. The formation of new rubber as the cause of the increase of the rubber-concentration.

In the above we have traced the circumstances under which the rubber-concentration of the latex increases. We have now to investigate on what this increase of concentration is based. An increase of concentration may be due to three causes; in the first place to the withdrawing of water from the latex, in the second place diffusion of rubber-particles from places where a higher concentration exists can diminish or neutralize existing concentration-differences, in the third place new rubber can be formed in the latex-vessels. The first phenomenon, the withdrawing of water from the latex actually takes place and may be demonstrated in Hevea under the following circumstances[1]). We have found (4), that when the latex flowing from a wounded Hevea is collected in successive parts and the osmotic value of each of them is determined by a determination of the freezing-point, in most cases the latex from the part flowing last, possesses both a lower osmotic value and a lower rubber-concentration. The latex remaining in the tree at the place of wounding after the flow must have been of a similar concentration. If the wound is reopened after some hours, both osmotic value and rubber-concentration of the latex appear to have considerably increased. This must be owing to the fact that the adjacent cells have drawn water from the latex.[2]) The question might be put whether in a resting tree identical circumstances may arise which cause the withdrawing of water from the latex-vessels. The volume of

[1]) In a recent publication on latex-flow (9) Zimmermann supposes, that an intensive formation of new rubber takes place near the wound. This supposition is not correct, since he has not noticed the phenomenon of with drawing of water from the latex-vessels by the adjacent cells.

[2]) I will discuss this process in a subsequent publication.

the laticiferous vessels will not be liable to great changes in a resting tree. To small changes the increase of the osmotic value of the latex we formerly found in some months of the year, points. Changes in the osmotic value of the latex however result in a relatively much slighter change of the volume of the latex-passages. This is due to the fact that the greater part of the volume is taken up by rubber. Because in the latex there are suspended so many rubber-particles which are not osmotically active, a slight change in volume of the latex-vessels will bring about a great change in the osmotic value of the latex[1]). If in the latex-vessels of a tree not in tap a continuous withdrawing of water occurred, we must find from root to top a regular in- or decrease of the concentration of all substances found in the latex. However the determinations of rubber-concentration and content of nitrogenous substances of the latex formerly made do not point in this direction. When after a long period of rest the rubber-concentration is examined in different places of the tree (2) we do not find that the concentration has increased in the spot where it was low, while in other places the concentration has decreased correspondingly, but the rubber-concentration has increased or attained its maximum in all places. The volume of the system of laticiferous vessels has rather increased through growth during that period, so that it must be accepted, that at any rate formation of new rubber has occurred. It might be however, that the formation of new rubber did not occur in the place, where the concentration has strongly decreased through tapping, but in other parts, because the phenomenon of local loss of water and subsequent flow of latex acts a part. We don't think

[1]) On account of this the system of laticiferous vessels is utterly unsuitable as a water-reservoir, as has been supposed by different authors. On the contrary we might talk in this case of an arrangement for the prevention of change of volume.

it possible to decide from the existing data whether such a loss of water takes place by the side of the formation of new rubber with the concentration-increase of the latex in trees not in tap, though the data indicate that this process certainly does not act an important part. On the second process that rubber can be equally distributed over the latex by diffusion we possess no data, but this influence, as will be demonstrated below, cannot be considerable either.

The third phenomenon, the formation of new rubber must, as we have seen above, take place in any case. The question only is whether this process acts the most important part in the increase of concentration of the latex in our experiments. There are various data pointing to this. In the first place we want to re-consider the behaviour of some individual trees with a view to this. In tree 62 we find the following values for the rubber-concentrations:

| May | June | July | August | Nov. | Dec. | Jan. | Febr. |
|---|---|---|---|---|---|---|---|
| 32.3 | 43.1 | 46.2 | 49.4 | 45.4 | 45.0 | 45.8 | 45.8 |

In the first three months the increase of concentration amounts to respectively 10.8, 3.1, 3.2 and in the second period of rest- 0.4, 0.8 and 0 %. In the first period therefore this tree behaves quite as the others and a continuous increase of concentration occurs. If such an increase of concentration was based upon loss of water and diffusion of rubber from other parts, it would be hard to understand, that in the second period there is nothing whatever to be observed of this phenomenon, and that then levelling of the concentration-differences in various parts of the system of laticiferous vessels does not occur at all. From the behaviour of the other trees we can practically draw the same conclusion, though the phenomenon is not so conspicuous in these. Only when we accept formation of new rubber in the latex-passages, it seems possible to us to explain, why there is now a strong increase of concentration in the first month now in the second or third. It may be imagined that the osmotic conditions of the tree are somewhat modified in some months e.g. during the wintering, yet this cannot possibly explain the lack of all increase of concentration during three months. We are therefore of opinion that though it is impossible to exclude the process of levelling of the rubber-concentration through diffusion and loss of water altogether, we may conclude from our data that this influence cannot possibly be very great. In the main *the increase of concentration must be owing to formation of new rubber.* On the nature of this process we will consider the various data.

### § 6. The factors, which influence the process of formation of new rubber in the latex-vessels.

In the first place the question may be put why laticiferous vessels from which latex is withdrawn, are producing rubber. The formation of the other constituents of the latex, viz. the substances determining the osmotic value and the nitrogenous substances proceeds rapidly as we explained in an other publication (2). If in a tapped tree the wound is re-opened next morning, the osmotic value and the concentration of the nitrogenous substances appears to have entirely recovered. The rubber-formation however progresses quite independently of these processes at a much slower rate. From our experiments in § 4 we drew the conclusion that the increase of concentration i.e. of the formation of new rubber, could not be due to the wound-stimulus. We see this process advancing at a various rate in various months, only then when the maximal rubber-concentration has been attained it comes to a standstill. *This has led us to the conception that if the conditions for this process are fulfilled, it will progress as long as it is not inhibited.* In a tree never yet tapped the laticiferous vessels are maximally filled and the formation of rubber is impossible. Only the latex-passages newly formed in the cambium can produce rubber. As soon however as on our opening the latex-vessels the rubber-concentration decreases and the other conditions necessary to this process, such as material from which the rubber must be composed and the enzymes required, are fulfilled, the formation of new rubber and the secretion of rubber-particles in the vacuole will take place. We have already mentioned that the rubber-formation does not come to a standstill in all trees at one and the same concentration. This may be due to various causes. We only point out the various sizes and shapes of the rubber-particles of different trees, which may influence the condition of maximal filling.

As to the strength of the formation of new rubber, it has appeared from our experiments, that it varies for one and the same tree in the various months of the year. It is obvious to think here of influences relating to the metabolism of the tree. Both wintering and the period in which the fruit ripens are important to numerous processes in the tree. Something of the great influence of those factors we see in the conversions and mobilisation of the starch in the bast, but most conspicuous is the influence exercised on the system of latex-vessels in the process of latex-flow. (2, 3, 4). *It is quite probable that these factors also determine the strength of the formation of new rubber.*

On the course of the process of formation of new rubber we have derived another important datum from our experiments (§ 4 ad 2). The formation of new rubber is stronger with a low rubber-content of the latex. It seems however more probable, that here we have not to deal with an influence of the rubber already present, but with the serum. In the serum we probably find the substances from which the rubber is formed by the protoplasm. *In that case it might be understood that according as more serum is present, the formation of new rubber progresses more rapidly.* About the nature of the substances in the serum

## TABLE III A.

| Nr. | Febr. | — | March | — | Apr. | — | May | Aug. | — | Sept. | — | Oct. | — | Nov. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35.7 | 7.6 | 43.3 | 1.5 | 44.8 | 4.5 | 49.3 | 36.8 | 8.9 | 45.7 | 7.7 | 53.4 | 3.4 | 56.8 |
| 4 | 33.3 | 8.3 | 41.6 | -0.5 | 41.1 | 5.2 | 46.3 | 32.3 | 5.6 | 37.9 | 10.3 | 48.2 | 3.5 | 51.7 |
| 9 | 38.5 | 8.4 | 46.9 | 0.8 | 47.7 | 1.8 | 49.5 | 38.4 | 8.8 | 47.2 | 5.9 | 53.1 | 1.9 | 55.0 |
| 12 | 35.5 | 10.1 | 45.6 | 1.8 | 47.4 | -0.2 | 47.2 | 30.9 | 11.1 | 42.0 | 8.1 | 50.1 | 1.4 | 51.5 |
| 15 | 35.2 | 9.6 | 44.8 | 1.1 | 45.9 | 2.5 | 48.4 | 40.8 | 1.5 | 42.3 | 2.5 | 44.8 | 8.6 | 53.4 |
| 40 | 32.3 | 12.9 | 45.2 | 0.6 | 45.8 | 6.8 | 52.6 | 30.5 | 3.0 | 33.5 | 11.0 | 44.5 | 7.3 | 51.8 |
| 41 | 34.7 | 7.2 | 41.9 | 1.5 | 43.4 | 1.8 | 45.2 | 41.2 | 2.3 | 43.5 | 0.1 | 43.6 | 6.3 | 49.9 |
| 43 | 41.1 | 6.6 | 47.7 | 1.0 | 48.7 | 0.7 | 49.4 | 40.2 | 4.6 | 44.8 | 6.7 | 51.5 | 2.5 | 54.0 |
| 61 | 37.1 | 5.4 | 42.5 | 1.6 | 44.1 | 6.9 | 51.0 | 38.9 | 0.6 | 39.5 | 1 0 | 40.5 | 5.8 | 46.3 |
| 70 | 33.9 | 7.4 | 41.3 | 2.5 | 43.8 | 4.1 | 47.9 | 30.8 | 1.4 | 32.2 | 17.6 | 49.8 | 5.5 | 55.3 |
| 71 | 43.6 | 6.4 | 50.0 | -0.1 | 49.9 | 1.1 | 51.0 | 44.2 | 1.9 | 46.1 | 8.1 | 54.2 | 1.3 | 55.5 |
| 78 | 39.1 | 3.9 | 43.0 | 2.3 | 45.3 | 1.4 | 46.7 | 40.3 | 0.4 | 40.7 | 10.0 | 50.7 | 4.3 | 55.0 |
| 79 | 40.0 | 5.2 | 45.2 | 0.1 | 45.3 | 5.1 | 50.4 | 36.4 | 7.1 | 43.5 | 3.6 | 47.1 | 6.7 | 53.8 |
| 81 | 38.0 | 5.7 | 43.7 | 1.6 | 45.3 | 3.0 | 48.3 | 32.3 | 7.0 | 39.3 | 7.0 | 46.3 | 4.7 | 51.0 |
| 89 | 29.7 | 8.9 | 38.6 | 1.2 | 39.8 | 5.0 | 44.8 | 34.1 | 4.0 | 38.1 | 10.5 | 48.6 | 2.3 | 50.9 |
| 99 | 34.8 | 7.3 | 42.1 | 2.0 | 44.1 | 3.2 | 47.3 | 33.8 | 8.3 | 42.1 | 9.1 | 51.2 | 4.3 | 55.5 |
| 101 | 33.4 | 6.4 | 39.8 | 2.3 | 42.1 | 2.9 | 45.0 | 29.9 | 1.8 | 31.7 | 14.4 | 46.1 | 3.4 | 49.5 |
| 118 | 37.3 | 6.6 | 43.9 | -0.1 | 43.8 | 1.8 | 45.6 | 40.0 | 7.4 | 47.4 | 0.9 | 48.3 | 2.6 | 50.9 |
| 119 | 35.0 | 6.5 | 41.5 | 2.7 | 44.2 | 4.4 | 48.6 | 40.1 | 0.9 | 41.0 | 6.8 | 47.8 | 2.1 | 49.9 |
| 121 | 28.4 | 4.8 | 33.2 | 2.8 | 36.0 | 2.3 | 38.3 | 26.4 | 7.0 | 33.4 | 10.9 | 44.3 | 2.2 | 46.5 |
| 138 | 31.8 | 6.3 | 38.1 | 2.2 | 40.3 | 7.5 | 47.8 | 33.4 | 6.9 | 40.3 | 10.6 | 50.9 | 3.2 | 54.1 |
| 148 | 37.3 | 9.5 | 46.8 | 1.0 | 47.8 | 4.0 | 51.8 | 35.7 | 10.5 | 46.2 | 6.8 | 53.0 | 3.8 | 56.8 |
| 157 | 40.0 | 5.6 | 45.6 | -0.4 | 45.2 | 1.1 | 46.3 | 37.0 | 5.3 | 42.3 | 6.9 | 49.2 | 3.6 | 52.8 |
| 158 | 32.8 | 7.0 | 39.8 | 4.0 | 43.8 | 9.7 | 53.5 | 37.8 | 0.3 | 38.1 | 11.0 | 49.1 | 6.1 | 55.2 |
| 159 | 33.9 | 5.7 | 39.6 | -.04 | 39.2 | 9.5 | 48.7 | 38.6 | 3.5 | 42.1 | 4.5 | 46.6 | 7.2 | 53.8 |
| 160 | 28.9 | 7.7 | 36.6 | 3.9 | 40.5 | 3.2 | 43.7 | 34.7 | 2.3 | 37.0 | 8.5 | 45.5 | 6.0 | 51.5 |
| 167 | 34.5 | 8.3 | 42.8 | -1.1 | 41.7 | 0.5 | 42.2 | 37.1 | 8.2 | 45.3 | 6.3 | 51.6 | -0.7 | 50.9 |
| 168 | 31.0 | 10.6 | 41.6 | 5.3 | 46.9 | 5.1 | 52.0 | 40.6 | 1.9 | 42.5 | 10.3 | 52.8 | 5.7 | 58.5 |
| 179 | 26.4 | 9.2 | 35.6 | 8.3 | 43.9 | 4.7 | 48.6 | 29.0 | 4.0 | 33.0 | 8.4 | 41.4 | 5.5 | 46.9 |
| 180 | 33.8 | 7.0 | 40.8 | 7.9 | 48.7 | 1.0 | 49.7 | 32.7 | 5.9 | 38.6 | 8.2 | 46.8 | 3.6 | 50.4 |
| 188 | 30.9 | 10.3 | 41.2 | 3.2 | 44.4 | 5.2 | 49.6 | 31.6 | 7.0 | 38.6 | 11.2 | 49.8 | 3.8 | 53.6 |
| 189 | 35.8 | 0.9 | 36.7 | 1.0 | 37.7 | 4.7 | 42.4 | 26.9 | 2.6 | 29.5 | 13.0 | 42.5 | 5.2 | 47.7 |
| 197 | 34.7 | 11.0 | 45.7 | -0.9 | 44.8 | 3.3 | 48.1 | 39.2 | 0.1 | 39.3 | 11.3 | 50.6 | 0.3 | 50.9 |
| 198 | 22.5 | 9.1 | 31.6 | 4.8 | 36.4 | 6.2 | 42.6 | 26.7 | 5.0 | 31.7 | 9.8 | 41.5 | 4.8 | 46.3 |
| 199 | 26.2 | 11.3 | 37.5 | 3.2 | 40.7 | 7.6 | 48.3 | 37.2 | 4.2 | 41.4 | 7.6 | 49.0 | 4.8 | 53.8 |
| 208 | 30.8 | 7.7 | 38.5 | 1.1 | 39.6 | 5.1 | 44.7 | 33.3 | 0.2 | 33.5 | 12.7 | 46.2 | 2.4 | 48.6 |
| 377 | 37.8 | 5.1 | 42.9 | 0.9 | 43.8 | 0.7 | 44.5 | 32.6 | 4.8 | 37.4 | 0.3 | 37.7 | 5.9 | 43.6 |
| 385 | 31.3 | 3.6 | 34.9 | 3.1 | 38.0 | 7.2 | 45.2 | 30.9 | 5.6 | 36.5 | 4.1 | 40.6 | 4.2 | 44.8 |
| 392 | 26.4 | 8.5 | 34.9 | 3.2 | 38.1 | 1.0 | 39.1 | 34.6 | 0.8 | 35.4 | 7.7 | 43.1 | 3.3 | 46.4 |
| 397 | 29.2 | 8.1 | 37.3 | 0.8 | 38.1 | 3.8 | 41.9 | 31.1 | 1.4 | 32.5 | 11.7 | 44.2 | 5.0 | 49.2 |
| Average | 33.8 | 7.5 | 41.3 | 1.9 | 43.2 | 3.9 | 47.1 | 35.0 | 4.3 | 39.3 | 8.1 | 47.4 | 4.1 | 51.5 |

Rubber-concentration of individual trees in three-monthly periods of rest determined at the beginning of each month.

TABLE III B.

| Nr. | May | — | June | — | July | — | Aug. | Nov. | — | Dec. | — | Jan. | — | Febr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 30.4 | 10.9 | 41.3 | 3.9 | 45.2 | 3.8 | 49.0 | 35.3 | 8.3 | 43.6 | 6.6 | 50.2 | 1.8 | 52.0 |
| 19 | 31.2 | 10.0 | 41.2 | 3.7 | 44.9 | 1.6 | 46.5 | 36.3 | 4.7 | 41.0 | 7.8 | 48.8 | 1.9 | 50.7 |
| 24 | 35.0 | 10.0 | 45.0 | 5.4 | 50.4 | 2.0 | 52.4 | 42.3 | 7.1 | 49.4 | 3.8 | 53.2 | 1.2 | 54.4 |
| 25 | 28.6 | 9.6 | 38.2 | 7.8 | 46.0 | 2.1 | 48.1 | 29.9 | 12.9 | 42.8 | 5.9 | 48.7 | 0.2 | 48.9 |
| 29 | 28.1 | 12.7 | 40.8 | 5.0 | 45.8 | 1.3 | 47.1 | 36.3 | 2.2 | 38.5 | 9.0 | 47.5 | 2.4 | 49.9 |
| 32 | 24.7 | 11.3 | 36.0 | 7.1 | 43.1 | 1.9 | 45.0 | 33.0 | 5.1 | 38.1 | 7.0 | 45.1 | 0.7 | 45.8 |
| 45 | 39.3 | 6.4 | 45.7 | 2.3 | 48.0 | 1.4 | 49.4 | 43.6 | 4.5 | 48.1 | 1.4 | 49.5 | 3.7 | 53.2 |
| 46 | 24.2 | 17.0 | 41.2 | 6.7 | 47.9 | 3.3 | 51.2 | 33.4 | 9.0 | 42.4 | 6.2 | 48.6 | 0.1 | 48.7 |
| 47 | 32.8 | 11.0 | 43.8 | 6.5 | 50.3 | 3.9 | 54.2 | 42.9 | 7.0 | 49.9 | 6.8 | 56.7 | -0.6 | 56.1 |
| 48 | 30.3 | 7.1 | 37.4 | 1.7 | 39.1 | 2.6 | 41.7 | 34.2 | 9.3 | 43.5 | 3.7 | 47.2 | 0.8 | 48.0 |
| 49 | 24.6 | 8.5 | 33.1 | 6.3 | 39.4 | 4.0 | 43.4 | 31.6 | 7.9 | 39.5 | 3.4 | 42.9 | -0.6 | 42.3 |
| 63 | 36.3 | 6.9 | 43.2 | 4.5 | 47.7 | 2.1 | 49.8 | 37.8 | 8.5 | 46.3 | 4.2 | 50.5 | -0.2 | 50.3 |
| 65 | 32.7 | 7.7 | 40.4 | 5.1 | 45.5 | 1.6 | 47.1 | 34.3 | 7.9 | 42.2 | 4.1 | 46.3 | 0.4 | 46.7 |
| 72 | 33.7 | 7.2 | 40.9 | 8.1 | 49.0 | 0.2 | 49.2 | 43.4 | 6.3 | 49.7 | 4.0 | 53.7 | 0.3 | 54.0 |
| 73 | 38.4 | 8.1 | 46.5 | 3.3 | 49.8 | 1.6 | 51.4 | 42.1 | 4.6 | 46.7 | 7.2 | 53.9 | 1.5 | 55.4 |
| 75 | 36.8 | 11.0 | 47.8 | 5.8 | 53.6 | 2.3 | 55.9 | 36.9 | 13.9 | 50.8 | 3.7 | 54.5 | 0.1 | 54.6 |
| 82 | 33.7 | 11.9 | 45.6 | 4.4 | 50.0 | 1.6 | 51.6 | 44.5 | 5.2 | 49.7 | 3.3 | 53.0 | 0.8 | 53.8 |
| 92 | 24.9 | 4.7 | 29.6 | 4.7 | 34.3 | 2.8 | 37.1 | 33.2 | 6.1 | 39.3 | 2.6 | 41.9 | 1.9 | 43.8 |
| 103 | 29.6 | 6.6 | 36.2 | 6.4 | 42.6 | 2.5 | 45.1 | 38.1 | 6.3 | 44.4 | 4.3 | 48.7 | 1.7 | 50.4 |
| 125 | 35.8 | 5.1 | 40.9 | 3.8 | 44.7 | 3.7 | 48.4 | 40.7 | 1.7 | 42.4 | 7.3 | 49.7 | 0.2 | 49.9 |
| 142 | 33.0 | 8.3 | 41.3 | 7.7 | 49.0 | 0.9 | 49.9 | 39.0 | 6.6 | 45.6 | 4.9 | 50.5 | 0.4 | 50.9 |
| 151 | 38.6 | 8.2 | 46.8 | 1.2 | 48.0 | -0.1 | 47.9 | 45.2 | 4.7 | 49.9 | 1.6 | 51.5 | -0.1 | 51.4 |
| 154 | 36.8 | 9.0 | 45.8 | 6.6 | 52.4 | 1.2 | 53.6 | 37.0 | 9.5 | 46.5 | 5.9 | 52.4 | 0.2 | 52.6 |
| 161 | 32.3 | 7.0 | 39.3 | 4.2 | 43.5 | 1.7 | 45.2 | 41.9 | 4.6 | 46.5 | 1.9 | 48.4 | 1.5 | 49.9 |
| 164 | 28.9 | 10.2 | 39.1 | 2.7 | 41.8 | 1.2 | 43.0 | 32.7 | 5.0 | 37.7 | 8.1 | 45.8 | 2.1 | 47.9 |
| 173 | 31.7 | 4.6 | 36.3 | 1.3 | 37.6 | 3.7 | 41.3 | 36.5 | 0.1 | 36.6 | 6.9 | 43.5 | 0.2 | 43.7 |
| 174 | 33.2 | 6.3 | 39.5 | 5.7 | 45.2 | 2.3 | 47.5 | 34.4 | 10.0 | 44.4 | 5.4 | 49.8 | 0.9 | 50.7 |
| 181 | 28.7 | 13.0 | 41.7 | 5.6 | 47.3 | 0.1 | 47.4 | 39.7 | 4.0 | 43.7 | 7.4 | 51.1 | 0.7 | 51.8 |
| 183 | 36.2 | 6.3 | 42.5 | 3.5 | 46.0 | 2.0 | 48.0 | 35.6 | 10.7 | 46.3 | 5.9 | 52.2 | 0.5 | 52.7 |
| 184 | 25.3 | 8.1 | 33.4 | 4.4 | 37.8 | 2.0 | 39.8 | 31.3 | 4.4 | 35.7 | 5.9 | 41.6 | 0.7 | 42.3 |
| 194 | 25.6 | 3.4 | 29.0 | 3.1 | 32.1 | 3.2 | 35.3 | 27.3 | 4.9 | 32.2 | 8.9 | 41.1 | 1.7 | 42.8 |
| 201 | 30.1 | 7.9 | 38.0 | 7.0 | 45.0 | 0. | 45.0 | 33.9 | 10.0 | 43.9 | 5.6 | 49.5 | 0.9 | 50.4 |
| 203 | 34.4 | 7.8 | 42.2 | 1.8 | 44.0 | 0.5 | 44.5 | 35.4 | 6.6 | 42.0 | 9.8 | 51.8 | 0.5 | 52.3 |
| 204 | 30.5 | 7.9 | 38.4 | 5.7 | 44.1 | 1.7 | 45.8 | 41.0 | 0.9 | 41.9 | 4.4 | 46.3 | 0.2 | 46.5 |
| 211 | 27.7 | 13.1 | 40.8 | 4.3 | 45.1 | 1.9 | 47.0 | 31.0 | 9.7 | 40.7 | 6.1 | 46.8 | 0.7 | 47.5 |
| 213 | 29.0 | 11.7 | 40.7 | 6.1 | 46.8 | 0.9 | 47.7 | 39.7 | 8.3 | 48.0 | 1.0 | 49.0 | -0.1 | 48.9 |
| 372 | 31.4 | 10.1 | 41.5 | 2.7 | 44.2 | 6.7 | 50.9 | 35.5 | 6.7 | 42.2 | 6.3 | 48.5 | 2.2 | 50.7 |
| 373 | 30.8 | 7.3 | 38.1 | 4.9 | 43.0 | 8.1 | 51.1 | 31.7 | 11.7 | 43.4 | 4.9 | 48.3 | 1.7 | 50.0 |
| 400 | 36.0 | 3.8 | 39.8 | 9.0 | 48.8 | 0.8 | 49.6 | 42.4 | 7.2 | 49.6 | 3.9 | 53.5 | 3.7 | 57.2 |
| Average | 31.6 | 8.6 | 40.2 | 4.9 | 45.1 | 2.2 | 47.3 | 37.0 | 6.7 | 43.7 | 5.3 | 49.0 | 1.0 | 50.0 |

which are to be considered in this process, we know but little. It is however important to point out that by the side of quebrachite other sugar-containing substances occur in the latex (van Dillen (7). In tapped trees Spoon (8) found that the content of these substances shows minima in the period of wintering and fruit-ripening. A closer investigation into the relation between these substances and the formation of new rubber seems most desirable.

Finally we have to discuss in how far the formation of new rubber in trees regularly tapped progresses in the same way as in trees in rest. The rate of that formation of new rubber cannot be determined so easily because in tapped trees the above mentioned phenomenon of drawing off water by the adjacent cells occurs, which renders the process of the increase of concentration much more intricate. It seems however very probable that the rules for the process of formation of new rubber found above also hold good for trees in tap. Only it might be, that in tapped trees there are additional factors influencing the rubber-formation. As one of those factors we may possibly regard the decrease of pressure which is due to the loss of latex and which though quickly stopped, prevails in the latex-vessels of tapped trees for some time. An influence of the wound-stimulus does not exist in our opinion.

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