RUBBER RESEARCH INSTITUTE OF MALAYA.

The Natural Coagulation of Hevea Latex

By

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It is well known that if an incision be made in the bark of *Hevea brasiliensis*, a viscous, creamy-white liquid, latex, exudes. In the course of a few hours after tapping, the latex begins to clot and eventually a solid mass of rubber is formed and floats on the surface of a serum which may be clear or of a milky colour.

The agencies by which the changes occurring during natural coagulation are promoted have been the subject of many investigations during the last two decades. Although progress was slow at the outset, rapid strides have been made in recent years and we appear to be in sight of a solution of the problem.

Formerly, two different lines were taken in explaining the phenomena and a not inconsiderable amount of evidence, although largely circumstantial, was adduced in support of the rival hypotheses. One body of workers considered natural coagulation to be the work of enzymes, while the other attributed it to bacterial activity. It is noteworthy that the supporters of enzyme action have had to shift their ground on more than one occasion. Later workers have shown fuller appreciation of the complexity of the subject and have displayed some reluctance in attempting an explanation of the subject in terms of any one theory.

**The Enzyme Theory.**

Although Spence (1, 2) announced the presence of an oxidising enzyme in Hevea latex over twenty years ago, it was in 1912 that Whitby (4) came to the conclusion that the natural coagulation of latex was due to enzyme activity. This view received the support of Campbell (9), as a result of work on the relation between calcium salts and coagulation, and of Barrowcliff (11), who discovered that coagulation proceeded in the presence of bactericidies, such as chloroform, but was inhibited by hydrocyanic acid, a substance known to be toxic to both bacteria and enzymes.

More recently de Vries and his co-workers (20, 21, 23, 25, 27, 28) have carried out extensive researches on the subject and consider that, while bacteria play a part in initiating the process, coagulation is finally effected by a specific enzyme, coalsae, which is present in Hevea latex.

Much of the evidence adduced in support of an enzyme theory of coagulation has not borne closer scrutiny. To take one ins-
stance, chloroform, toluene and thymol are now known to be without toxic action on latex bacteria.

The outstanding points in favour of this theory, which have not yet been completely explained on other grounds are listed below:

(i) Latex which has been sterilised (the enzyme presumably destroyed) does not coagulate on standing or in presence of acid, but does so after addition of a small quantity of fresh latex.*

(ii) Coagulation is accelerated by addition of calcium salts, which are known to favour clotting enzymes, but inhibited by calcium precipitants such as oxalate and fluoride.†

(iii) Campbell (9) and Whitby (12) have pointed out the analogy existing between latex coagulation and the clotting of blood and of milk respectively.

THE BACTERIAL THEORY.

The first attempt to explain the coagulation of Hevea latex on a bacteriological basis was made by Eaton and Grantham (6). These investigators launched certain criticisms at the enzyme theory and, as a result of their researches, concluded that natural coagulation is due to the activity of certain bacteria which infect the latex after collection.

The views of Eaton and Grantham received considerable support from the work of Gorter and Swart (7) who found lactic acid present in the serum after latex had been coagulated in presence of sugar. Gorter and Swart considered that lactic acid was a result of the presence of fermenting organisms and other organisms were responsible for the putrefactive changes occurring at the surface of the medium.

A bacteriological study of Hevea latex was carried out by Dernier and Vernet (10) and their results are of considerable interest. Although 27 different bacteria were isolated by these workers only one of them, "Bacillus No. 1", was present in all samples examined. "Bacillus No. 1" was distinctive also in being the only organism found which was able to ferment "dambosite"—the latex "carbohydrate".

At a later date, Belgrave (18) summarised the situation and gave further experimental evidence in favour of the bacterial theory.

*This statement, first made by Barrowcliff, appears, in general, to be true although Eaton and Grantham (6) and Belgrave (18) have refuted it. van Harpen (29) now offers another explanation.

†Belgrave (18) has weakened the case for the enzyme theory by pointing out that sulphate (not a calcium precipitant) also inhibits coagulation
The points in favour of the bacterial theory are:

(i) Latex collected under sterile conditions remains liquid and latex sterilised by heat does not undergo coagulation until exposed to fresh infection.

(ii) In his attempts at collecting sterile latex, Belgrave found bacteria present in latex which coagulated but none in latex which remained liquid.

(iii) Of the bacteria found in latex, one species fermented the latex "carbohydrate" with production of acid and this bacillus was present in all samples examined.

THE WORK OF DE VRIES AND BEUMÉE-NIEUWLAND.

de Vries and Beumée-Nieuwland consider* that the stability of latex is dependent on two factors, (i) the electric charges on the rubber particles and (ii) solvation.

Rubber in latex is a negatively charged colloid and has a low degree of solvation. Consequently flocculation of the particles can be brought about either by neutralising the charges with positively charged ions or by the agency of a dehydrating agent such as alcohol. It is well known, of course, that addition of acid or alcohol to latex results in coagulation, but alcohol is not able to effect precipitation of the hydrocarbon particles after the serum salts have been removed by dialysis.

Under ordinary conditions separation of the rubber particles may occur by

(i) creaming: a purely mechanical and reversible change due to the action of gravity;

(ii) flocculation: in which the separated rubber particles do not adhere and can be temporarily redispersed by shaking. Flocculation is brought about by addition to latex of an electrolyte which causes unloading of the charges to such an extent, that the mutual repulsion of the now feebly charged particles is more than counter-balanced by the molecular attraction existing between them;

(iii) coagulation: the stage following flocculation and in which the flocculi unite to form a coherent clot.

Natural coagulation is the result of bacterial activity. The latex bacteria attack certain carbohydrate-like compounds present, acids are produced and the pH value of the medium is lowered. When the pH value has dropped to about 5.0, flocculation of the rubber particles occurs and the process of coagulation is completed by the enzyme, coagase.

* See Kruyt and de Jong, Zeit. physikal Chem., 1922, 100, 250.
Latex containing the bacteria and no enzyme (sterilised latex inoculated with bacteria) can reach only the flocculation stage: while latex containing only the enzyme (latex collected from the tree under sterile conditions) undergoes neither flocculation nor coagulation. Latex in which the enzyme alone is present, however, retains the power to coagulate latex which has already reached the flocculation stage.

The ability of acids to produce coagulation in latex is affected by heating, the more dilute the latex solution the higher the temperature to which it must be raised before this power is destroyed. After a 10 per cent. solution of latex has been heated to 75°C. for a few minutes, it can no longer be coagulated by addition of acid but, with a 33.3 per cent. solution, the same result is attained by heating to 65°C. Thus, inoculation of B. mixture with bacteria results in flocculation and not coagulation, although the rubber particles may eventually cohere mechanically; acetic acid also can produce only flocculation and not coagulation.

de Vries and Beumée-Nieuwland point out that the so-called “acid gap”† in latex is simply an example of the phenomenon of irregular series, well known with other colloid systems;‡ and is due to the discharge of the charges on the colloid particles by electrolytes and reloading with charges of the opposite sign. Enzymes do not necessarily play any part in it.

As emphasised by de Vries and Beumée-Nieuwland, latex containing neither enzymes nor bacteria is probably changed latex.

**The Present Investigation.**

Samples of latex of various ages were examined and, in all cases where coagulation had begun, the samples were found to be swarming with bacteria. Cultures of organisms were prepared and a bacillus was isolated without difficulty. This organism was the predominant feature of the latex flora and was invariably present in all samples examined from the States of Selangor, Perak and Johore.

The bacillus has been studied in considerable detail and appears to be responsible for most of the important biochemical changes

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*10 per cent. latex solution maintained at boiling temperature for a few minutes.
†While addition of acid to latex results in coagulation, the initial addition of a greater quantity may not cause precipitation; further addition of acid, however, will again result in coagulation. van Harpen has recently determined the pH values of latex at which these changes occur.
‡See, for example, the flocculation of a platinum sol by ferric chloride. (Buxton and Teague, Zeit. physikal Chem., 1904, 57, 72, & 79.)
which occur in latex during natural coagulation. It does not appear to have been denominated previously and the name *Bacillus pandora* is proposed for it.

Cultures were prepared on glucose-agar slopes and sub-cultures were made at weekly intervals. The characterisation of the organism is given in detail on page 10 but some of the characters and reactions are of particular interest.

The organism is rather large and, as it stains readily with carbol-fuchs in, there is little difficulty in identifying it in mixed cultures. It is motile and the flagella admit of staining by Loeffler’s method. It is Gram positive, contrary to the statement of Dernier and Vernet who found their “*Bacillus No. I*” to be Gram negative. *B. pandora* is a spore former and a facultative anaerobe† and, in consequence of the latter characteristic, is able to effect chemical changes both in the liquid and at the surface.

Gelatine is liquefied by *B. pandora*, nitrates are reduced and litmus is rapidly decolorised. Acid formation occurs in the presence of sugars and of milk but only in the case of lactose and sucrose has gas evolution also been observed.

Chain formation was not marked but appears to be more noticeable in older cultures. The cultural features were not distinctive, the colonies on both agar and glucose being punctiform. Tests for the production of hydrogen sulphide gave negative results.

Inoculation of solutions of latex and latex products with *B. pandora* gave interesting results.

Addition of the organism to sterile 10, 20, and 33.3 per cent. aqueous solutions of latex produced flocculation in 2 or 3 days. In some instances, coagulation was observed after two or three weeks but the tendency towards formation of a coherent clot varied from sample to sample. It can be stated, however, that coalescence is favoured by a high concentration of latex in the solution. Acidification without gas evolution resulted from inoculation of latex serum and of a solution of the so-called latex “carbohydrate”, (methyl-1-inositol) prepared from latex by extraction with alcohol.

Experiments showed that the pH range of *B. pandora* is wide, the organism being detected in solutions whose pH values ranged between 4.2 and 10.2. Ammonia *per se* does not appear to have any toxic properties whatever and sodium sulphite, in the concentration in which it is usually employed as an anti-coagulant, is also without bactericidal action.

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*See Belgrave, Malayan Agric. J., 1923, 11, 351.*

†Major B. J. Eaton informs me that Dr. Stanton, formerly Bacteriologist and later Director at the Institute of Medical Research, Kuala Lumpur, isolated an organism from Hevea latex which proved to be a facultative anaerobe. The work has not been published.
It seems highly probable that the "Bacillus No. 1" of Dernier and Vernet is identical with *B. pandora*, and a comparison of characters is given below.

<table>
<thead>
<tr>
<th>Character</th>
<th>Bacillus No. 1</th>
<th>B. pandora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex coagulation</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Acidification of &quot;damboseite&quot;</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Acidification of lactose</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Acidification of sucrose</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Coagulation of milk</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Albumen digestion</td>
<td>...</td>
<td>-</td>
</tr>
<tr>
<td>Gelatine liquefaction</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Gram stain</td>
<td>...</td>
<td>-</td>
</tr>
<tr>
<td>Motility</td>
<td>...</td>
<td>-</td>
</tr>
</tbody>
</table>

It will be seen that the experimental results wholly support the bacterial theory of natural coagulation and the mechanism by which the stage of flocculation is reached is in accord with the views of de Vries and Beumée-Nieuwland.

Latex, as it issues from the tree, is almost certainly free from bacteria for latex removed from the tree under sterile conditions remains liquid.

The fact that *B. pandora* is a spore-former indicates that it is to be found as spores on the tree trunk and enters the latex as it issues from the tapping cut. On a wet morning, the tree trunks are moist and, under such favourable conditions, the spores germinate. Thus, in addition to contamination at the tapping cut, active organisms will be carried to the latex flow by the rain running down the trunk. It would be anticipated that latex would coagulate more rapidly on wet mornings and such is known to be the case.

When the bacilli reach the latex the carbohydrate substances are attacked and acids are produced.

Carbohydrates are always attacked in preference to other substances, as was realised clearly by Eaton and Grantham, for they showed that natural coagulation is accelerated by the addition of sugars to latex. The matter has also been discussed by Whitby (13). Decomposition of the proteins sets in only when most of the carbohydrate substances have been removed. When the latex has attained a definite degree of acidity (a pH value of about 4.8 according to van Harpen) flocculation and coagulation of the rubber particles take place.

At this stage the rubber particles with adhering protein float to the top of the liquid and other changes begin. Possibly the organisms in the serum are now being killed by the acid they have themselves produced and only those at the surface can survive; but, in any case, the separated rubber has carried bacteria with it.
to the surface and there decomposition of the adhering proteins sets in. As a result, ammonia and probably other reduction products appear and the surface layer becomes alkaline. Hence, in coagulated latex, the liquid and the surface clot are found to be acid and alkaline respectively. Both acid and alkali production can take place, of course, in the liquid or at the surface, provided the necessary carbohydrate or protein substances are present.

Whether rubber particles in a state of flocculation are coagulated by an enzyme or a resinous substance is a matter beyond the scope of this paper.

In none of the experiments carried out was the surface scum evil-smelling or badly discoloured and it does not appear likely that these results are produced by *B. pandora*.

### THE PRESERVATION OF LATEX.

Substances in use at present as latex preservatives fall into two categories:

(a) those employed to delay coagulation in the latex cups, known as "anti-coagulants;"

(b) those used to preserve latex in the liquid state for an indefinite period during export, known as "preservatives".

All that is required of substances in the first class is that they delay bacterial activity for a few hours; it is not necessary that all organisms present should be destroyed. In Malaya, sodium sulphite is generally employed for this purpose and, for estate practice, Eaton* recommends the use of a solution which gives 0.05 g. \(Na_2SO_3\) per 100 c.c. latex. In the present investigation it was found that *B. pandora* was able to multiply in solutions containing four times this quantity of sodium sulphite.

The success of sodium sulphite as an anti-coagulant is to be attributed to the fact that dilute solutions of lactic acid are able to decompose sulphite with evolution of sulphur dioxide and formation of sodium lactate. In this manner, accumulation of acid in the latex is avoided and coagulation cannot take place until all the sulphite present has been decomposed. The anti-coagulant action of sodium carbonate admits of an explanation along similar lines.

It may be pointed out that, with the quantities of sodium sulphite usually employed on rubber estates, the concentration of sulphur dioxide is not sufficiently high for this compound to have a marked toxic action on the bacteria present. Its effect, if any, in this direction is very slight.

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* Rubber Research Institute of Malaya, Planting Manual, No. 1 (1928), on page 3, line 35, "5 fluid ozs." should read "10 fluid ozs."
Formalin also has been employed as an anti-coagulant, probably on account of its well-known germicidal properties but, as its presence in excess has detrimental effects on the rubber, its use has been discontinued.

Ammonia in dilute solution has met with a measure of success as an anti-coagulant and its action is obviously to neutralise the acids as they are formed so that the pH value of the latex does not reach that at which coagulation takes place.

As a preservative during export the use of ammonia is general, although in the present investigation it has been found that ammonia is without toxic properties as far as B. pandora is concerned. Of course, if the ammonia added is sufficient to raise the pH of the latex to a value outside of the range of the organism, the bacterial activity will be destroyed; but this effect would be produced by any other strong alkali.

In actual practice, the quantity of ammonia added is not sufficient to raise the pH value to the required figure, hence the micro-organisms are not destroyed.

Eaton (17) recommends the use of 7 grams of ammonia (NH₃ by weight) per litre of latex for preservation for shipment while de Vries (19) considers 5 grams per litre to be the minimum for good preservation. The presence of four times the latter quantity, however, will not ensure the complete absence of micro-organisms. de Vries has shown that ammoniated latex exhibits a decrease in alkalinity on keeping although, in 0.25—0.3 per cent. solutions, the diminution is so slow that the latices keep well for over a year.

The action of ammonia as a preservative admits of an explanation as follows. The carbohydrate substances in latex are decomposed by bacterial action with production of organic acids, which, however, are neutralised by the ammonia as soon as they are formed. After decomposition of the whole of the acid convertible substances, the proteins are attacked and ammonia production becomes the predominant action. During this stage there is a loss of ammonia by evaporation and a gain by bacterial activity but, when the protein is decomposed, the ammonia concentration remains constant, except for the losses by evaporation, while the bacteria may die from starvation or attack the lactates, etc. formed. It will be clear that if the initial concentration of ammonia is not sufficient to neutralise the acids formed then coagulation will ensue eventually.

With the information available at present, it is not possible to evaluate accurately the acids produced by bacterial action but a calculation has been made which throws some light on the subject.

According to Pickles and Whitfield (3), smoked rubber may contain as much as 2.5 per cent. methyl-l-inositol. Assuming a