MODE OF OCCURRENCE OF CAOUTCHOUC IN THE GUAYULE, 
*PARTHENIUM ARGENTATUM* GRAY, AND ITS FUNCTION

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(WITH ONE PLATE)

The account which I published in 1911 (9) of the mode of occurrence of caoutchouc in guayule, *Parthenium argentatum* Gray, is incorrect. I was led into error by inadequacy of both method and point of view. The purpose of the present paper is to set the matter right, so far as I now understand it.

In the guayule, as in some other rubber-bearing plants, the rubber occurs in the parenchyma cells and is thus segregated. In contrast with this condition is that in the so-called latex-bearing rubber plants, such as *Hevea, Manihot, Ficus, Euphorbia, Scorzonera, Chondrilla*, etc., in which the rubber is a constituent, more or less prominent according to the species, of a white or colored milky fluid, which is stored in tubes from which, when opened, the fluid flows more or less freely. The fluid in question is always a complex emulsion of rubber¹ and other substances (resins, fats) as the internal phase, with water containing proteins, salts, sugars, etc., in solution as the external phase. The size of the suspenoids differs greatly, and is from 2 microns and less in *Hevea* and *Kickxia* to 60 microns or more in *Musa*. This general statement may now be extended to the guayule for, as will be shown, the rubber associated with other substances occurs in the same manner. The fluid here is equally a latex² confined to individual cells. In *Hevea*, etc., it occurs originally in individual cells which break down in series to form tubes, or, as in the seed, in cells which tend to form inarticu-

¹ Latex is, of course, not always rubber-bearing (see Molisch, 14; Lloyd, 12).

² The word "latex," while meaning merely a "fluid," has come to be used more specifically for milky rubber-bearing fluids occurring in tubular structures (Oxford Dictionary). It is, however, impossible to adhere to this usage rigorously. For example, the milky exudate from cut surfaces of *Musa* is always spoken of as latex in the more restricted sense; as a matter of fact, this latex is merely the sap (latex in the original sense) of large barrel-shaped cells placed in series like a string of beads, each cell separated from its neighbor by a thin circular wall (double, of course). On cutting fresh living tissue, the internal pressures break down the walls, hence the exudate. If the part cut is fully wilted or has otherwise lost its turgor (as when a banana fruit has been "chilled") (Lloyd, 11) no exudation takes place. The word "latex" is here obviously misapplied unless used in its primary sense. I should prefer, therefore, to adhere to the primary meaning, coupled with qualifying terms, as parenchyma latex (*Parthenium, Musa*), vascular latex (*Euphorbia, Hevea*). It is well understood the latex vessels of *Hevea* arise by the resorption of the contiguous walls of seriate members. "Sap" could be restricted to the fluid of wood vessels.

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late tubes (Parkin, 15), such as those which occur in many other plants, e. g., Argemone (Trecul, 18).

The evidence as to the nature of the parenchyma latex of guayule is very simple. If a living plant stem be cut across and the cut surface be dipped into a drop of water on a glass slide and examined at once, the water will be observed to have become milky. Placed under the microscope, it is seen to be a rich suspension of minute particles in lively Brownian movement. If now a section be cut, thick enough to afford uncut cells but thin enough so that uncut cells do not overlie each other, and examined microscopically, the contents of the cells appear as a densely milky fluid, more especially if observed in reflected light with the dark field condenser. In transmitted light, the contents of the cells appear cloudy and more or less obscure, according to their density. At the same time, one may observe that the minute particles or suspensoids in the fluid in each cell are in Brownian movement, the observation of which is most easily made in cells which are not too fully packed with the suspensoids, for obvious reasons. A series of preparations made from a shrub containing poorly to richly loaded cells will afford a variety of pictures, from cells containing only a few minute suspensoids to those containing a very dense suspension. Such preparations, if photographed with a time exposure, give a curious result pictorially, which of course could be expected, namely, cells appearing as if filled with an ill-defined cloud (plate II, figs. 1, 3). It is only when a rapid exposure is used that the individual particles may be resolved, for which purpose not too well filled cells are best (plate II, fig. 5). An instantaneous photograph of diluted cell fluid affords a picture in which one may obtain an impression of the range of size of the particles, which extends from two microns to those of so minute a size as to be just within the limits of resolvability (plate II, fig. 8). The particles are all spherical, as in the latex of Ficus, and not pear-shaped, as are many of the suspensoids of Hevea. It is not, of course, to be supposed that all the particles are caoutchouc in guayule any more than in other rubber-bearing plants. But that a part of them, indeed the greater part, is caoutchouc may be proved by the use of suitable solvents for other possible components, such as resins, proteins, oils. Sections treated with such solvents for sufficient periods of time still reveal suspensoids remaining undissolved, provided only that a solvent for caoutchouc has not been used. If, however, for such tests cells are used which contain a great deal of rubber, this will have become coagulated. If, on the other hand, cells with a small amount are used, the particles may remain discrete though they usually become more or less adherent to various surfaces present—the mere result of space relations.

If now coagulating agents are used at once, the coagulation of the latex within the individual cells proceeds and there results within each cell sev-
eral coagula if the amount of rubber is small—that is to say, flocculation takes place (plate II, figs. 4, 6); or, if there is enough rubber, a single clot is formed (plate II, figs. 2, 7). This, as is well shown in the figures in my monograph (1911), is not homogeneous internally, but contains inclusions of fluid—are vacuolated, so to speak—and particles of non-rubber constituents (plate II, fig. 7). The change is irreversible.

The above described behavior in the guayule is of interest both theoretically and practically. From the point of view of physiology it was difficult to regard the caoutchouc, if laid down in the parenchyma cells as solid masses, as a food or other reserve. The question of the function of latex, or, to particularize, of any or all of the constituents of the latex, has puzzled every one who has taken any theoretical interest in rubber plants, for the reason, if for no other, that if any or all of these constituents are physiologically useful, there might be expected some variation in the composition of the latex at one time or another in the cycle of growth, let us say, and thus during the year the crop of latex would be uneven in quality or quantity or both. While in the case of Hevea there seem to be minor differences in composition of the latex as between tree and tree, or more obviously between race and race, the differences are slight and appear to indicate nothing in regard to physiological significance. There is also the now known effect of enrichment of the soil by sodium nitrate, etc., in increasing the yield of latex (Grantham, 6), but it can be inferred that this effect is general, affecting many if not all features related to growth. Possibly the number of stone-cells in the cortex would be affected, but this feature is so different quantitatively in various races of Hevea that it would require hardihood to claim a physiological function for the stone-cell. Some would perhaps argue for a protective effect, but there is no evidence that this is the case: just as a similar effect has been argued for latex (de Vries, 19) in the sealing of wounds, without any real evidence that Hevea is less subject to the ills of the flesh than other non-latex producing plants. However, it may be suggested that the coagulative capacity of rubber and other latices serve for the prompt closure of wounds so that the internal pressures, which are high (Molisch, 14) may be restored and preserved.

As to the experimental evidence in support of the idea that latex is in part at least a fund for energy supply, there is the evidence adduced by Fäivre (3, 4, 5) for Tragopogon porrifolius, Morus alba, and a Ficus. By varying conditions (including absence of carbon dioxide) he observed impoverishment of the latex in the above forms, and inferred their nutritive value. His views were favorably received by de Vries (l. c., 19). Ch. Bernard (1) also did experiments with Euphorbia thyrsifolia and E. splendidens exposed to CO₂-free air and observed the corrosion of the starch grains; and what happens to one component of the latex may happen to an-
other. Pfeffer (16), however, asserts that among other substances India rubber is "incapable of further metabolism," which remark, whether eventually shown to be true or not, serves to indicate the crux of the problem for us at the moment. The only additional experimental evidence that is available is contained in a note by D. Spence (17), who states that in a young Ficus elastica "grown in an atmosphere and soil free of carbon dioxide, gradually drew on their milk which became nothing more than water after a few weeks' time." This experiment, supporting the conclusion of Faivre (3), indicates that in the absence of a raw material for the elaboration of a hydrocarbon, here caoutchouc, the supply already present may be metabolized. The presence of suitable enzymes, shown in the first instance by Spence (17) for Hevea and Funtumia, coupled with the great surface area of the suspensoids, lends support to the idea. The very condition in which the latex occurs, namely, in a vascular system, or system of tubes, their very wide distribution in the plant kingdom, and the evident nutritive values of many of the latex constituents predispose only to the view that, in general, latex is not merely a depository of wastes.

In considering the guayule and other plants which have and hold their caoutchouc in like manner, namely, in the individual cells of the parenchyma, the predisposition is to compare this condition to analogous ones in which known metabolically useful materials are accumulated, e. g., starch in the potato tuber. In the latter it is easy to demonstrate experimentally that the starch is actually and measurably used. Now that we know that the caoutchouc occurs as a colloidal suspension, precisely as in the latex of Hevea, etc., we have no difficulty in accepting the possibility of its attack by enzymes: what seemed difficult, holding the older and incorrect idea that the caoutchouc is disposed in solid masses, a single one in each cell, is now easy when the surface relations inherent in a colloidal suspension are realized (Molisch, 14, p. 80). On general principles there is no particular difficulty in expecting even so refractory a substance as rubber to be acted upon readily if the proper agencies (enzymes) are present. This would be merely the reversal of the anabolic process. This argument has been used by Molisch (l. c.) in regard to the particulate condition of the other substances in latex.

That, however, the hydrocarbon in question is, in the case of the guayule, actually a reserve substance and is as actually made use of in metabolism requires proof, which has not yet appeared. We know that during periods of rapid growth very little caoutchouc is formed, this happening after the cessation of growth during the drought period following the rain which made growth possible. The amount of rubber which can occur in maximum quantity (ca. 20 per cent.) depends not alone on the particular race of guayule (McCallum, 13), but also on the rapidity of growth and the length
of the drought following cessation of growth. At all events, in the semi-arid deserts in which it occurs, the guayule accumulates rubber until some maximum is reached and the amount then appears to remain constant until a new growth period sets in. The addition of new tissues during the growth period makes it very difficult to determine whether meanwhile the caoutchouc is being drawn upon as a source of energy. The solution of the problem is one of the most interesting in the field of plant metabolism and it is to be hoped that those favorably placed and interested will find it possible to find the solution.

The view that the caoutchouc in the guayule confers on the plant some ability to withstand drought has been advanced from time to time, but no convincing evidence has accrued. That the plant wilts at once on being pulled suggests a negative answer. On the other hand, plants so situated that they must withstand a drought period are subjected to a very gradually effective drying out which might affect the leaves without affecting the stems, while these may have a superior drought resistance conferred on them by the presence of the caoutchouc. The fact that it requires a considerable period of time after the shrub has been "pulled" before it "cures" so that it can be milled might be taken as an indication that during some period—probably a short one—the parenchyma cells retain their vitality as indicated by the dispersed condition of the contents of the cells.

On the other hand, closely related species, such as the mariola (Parthenium lyratum), which appear to be equally able to withstand drought, if not more so, have very little caoutchouc; but here we impinge on one of the very difficult problems in the field of adaptation, the question of how far the same ends are attained by different means.

An additional suggestion is prompted by the recent work of Walter (20), who reports that drought resistance of leaves (the stems were not investigated) "varies directly with the maximum osmotic value and with the osmotic inertia of the plant," namely, that the accumulation of caoutchouc and water-soluble substances such as oils and some resins may increase the osmotic value of the sap merely by occupying space, thus by indirect means gradually changing this from a lower value during the continuation of drought.

The practical interest attaching to the matter of the condition of the caoutchouc is well understood by those who during the past twenty-eight years have been concerned in the extraction of rubber from the guayule shrub. It has long been known in northern Mexico that by merely chewing the stems, rubber ("hule") could be obtained, and when communal methods were used a sufficient amount could be obtained to make balls for playing games. Rubber was well known to the prehistoric peoples of Mexico, who made small images, utensils and balls, etc. It was obtained
by coagulating the latex of *Castilloa*; and it is not unlikely that such objects found their way as far north as southern Arizona (Lloyd, 10). It is, however, pretty certain that in northern Mexico the rubber of guayule was also used (Lloyd, 9). At any rate, the method of obtaining small quantities by mastication is and has been general knowledge among the peons of northern Mexico for a long time, certainly previous to the beginnings of the manufacture of guayule rubber just previous to 1903. It was in this year that Mr. William A. Lawrence discovered that this substance could be extracted from the shrub by a strictly mechanical process of comminution and flotation in water, by which the floating rubber could be separated from the bagasse (Carnahan, 2). For many years thereafter, however, there were measurable and even serious losses due to various imperfections in the methods of manufacture, following from imperfect knowledge of what constituted "curing" the shrub, which we now know to be the time and conditions for bringing all the suspensoids into the coagulated condition. With the fact before us that the rubber in the guayule exists as a colloidal suspension, experimentation can proceed with a perfectly definite criterion before the investigator. It is one so simple, moreover, that it is a matter of surprise that it was not long ago discovered, namely, the method indicated above. One needs but to make a cut across the tissues and touch the cut surface to a drop of clean water on the piece of glass. If the rubber exists as suspensoids, they will flow out and render the water milky. Slight turbidity may be determined by putting a few slices of tissue in a small volume of water and then determining the turbidity by means of a strong beam of light (Tyndall effect). For the purpose of checking this method of treatment, the dried material, after allowing a drop to dry on the glass, may be treated with acetone to remove the resins, and the adhering caoutchouc then examined, when, of course, staining may be used.

At this point I wish to acknowledge the courtesies extended by Mr. George H. Carnahan, President of the Inter-Continental Rubber Company, who made it possible to re-examine living guayule, both at Salinas, California, and at Torreon, Mexico, in 1926 and 1927. It is with his concurrence that the above notes are published.

3 Since such plants as *Scorzonera* and *Chondrilla*, certain species of which are being exploited by the Russians, are vascular latex plants, and are hardly susceptible of being tapped, the latex in them must be allowed or caused to coagulate, before extraction, thus following the method of "pilonage" used in Africa for the extraction of the rubber from *Landolphia heudelotii* (Jumelle, 7); that is, by a method fundamentally similar to that used for guayule.
LITERATURE CITED


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EXPLANATION OF PLATE II

Fig. 1. Section of the living cortex, including a portion of a resin canal, of Parthenium argentatum Gray. The cells appear as if filled each with a dense cloud. This is the result of a time exposure on many suspensoids in Brownian movements. Compare with figure 5.

Fig. 2. The same section after brief treatment with weak acetic acid. The latex within the cells has now been coagulated in nearly solid masses, almost filling them.

Fig. 3. Living cells from the pith, time exposure. The cloudy appearance of the interior of the cells is owing to the movement of the suspensoids, as in figure 1.

Fig. 4. The same section as in figure 3, after treatment with acetic acid. The amount of material in the form of suspensoids is not great enough to make a solid mass, but is merely flocculated. Contraction follows later.

Fig. 5. Instantaneous photograph of three undamaged cells of the cortex, showing the suspensoids of caoutchouc (for the most part). A time exposure would have yielded a result such as in figures 1 or 3.

Fig. 6. The same after treatment with acetic acid.

Fig. 7. A larger picture of such cells as are seen in figure 2 showing that after coagulation the coagula are not homogeneous.

Fig. 8. Dark-field photograph of the parenchyma latex diluted with water.
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