new antigenic character conferred upon the individual at the time of conjugation persists in its progeny for at least one month of active growth. About 5 per cent. of all recent ex-conjugants seemed to be insensitive to sera which were effective on the cells with which they had mated.

Serologic tests done on pairs of animals while in conjugation have given variable results: in not a single instance have both members of pairs reacted in either antiserum after short periods of conjugation; in many instances both members of pairs have reacted in the antiserum in which they were tested after long periods (12 hours at 28° C. to 40 hours at 19° C.) of conjugation.

All conditions and observations of these experiments make it clear to the authors that the antigen involved in the reaction is very largely, if not exclusively, cytoplasmic in character. We therefore are of the opinion that during the course of conjugation in *Paramecium bursaria* there occurs an extensive interchange of cytoplasm, although it is admitted that the proper explanation of the reactions observed may relate to an alternative suggestion that the cytoplasm of paramecia undergoes a sudden and profound antigenic reorganization with reference to, and influenced by, the cytoplasm of the contiguous animal or the wandering pronucleus received from it. (The opinion that cytoplasmic interchange occurs was strengthened somewhat in finding, on more than one occasion in these experiments, zoochorellae in the conjugant which was previously free of these inclusions. However, this observation can not be taken as conclusive evidence, for it is a fact that under the influence of effective antiserum the conjugating animal is not normal externally, although it may be almost so in its internal organization.)

The position taken here with regard to cytoplasmic interchange during conjugation in paramecia apparently does not conform to the hypothesis held generally by zoologists since the work of Maupas[3] in 1889. An inextensive search through the literature in this field—an admittedly unfamiliar one to the authors—indicates that the only evidence against the occurrence of cytoplasmic interchange in conjugating paramecia is the fact that it has not been observed microscopically. At the turn of the present century Hickson[4] pointed out that this fact should be attributed to an inadequacy of the methods used and should not have been developed into the conclusion that interchange does not occur.

JAMES A. HARRISON
ELIZABETH H. FOWLER

DEPARTMENT OF BIOLOGY,
TEMPLE UNIVERSITY

[3] E. Maupas, *Arch. de Zool. Exp. et Gen.*, (2), 7: 149–517, 1889.

[4] Sidney J. Hickson, *Quart. Jour. Micros. Sci.*, 45: 346, 1902.

THE COAGULATION OF LATEX

THE mechanism of the coagulation of latex is a very brief chapter in most treatises on rubber. The theories are numerous and conflicting. The most widely accepted interpretation is one in which the destruction of the stabilization membrane is postulated. Recently, in Haiti, I made an electrophoretic study of latex involving the determination of mobility rates and isoelectric points. The timing of the migration of individual latex particles obviously necessitated the use of a microscope. This permitted constant observation of the globules, so that not only were their rate and direction of movement observed, but their aggregation as well.

When latex is put into a buffer mixture of a pH value at or near the isoelectric point of the latex, agglutination of the particles takes place. While observing this incipient coagulation of the latex, I was led to the conclusion that though pH values of isoelectric points indicate a protein covering on latex particles, electrophoretic behavior points to a surface which is, in part, non-protein. The very feeble charge on Cryptostegia latex globules in comparison with the greater charge on Castilloa and the still greater charge on Hevea suggests that there is least protein on the Cryptostegia particles, and most on the Hevea particles. This deduction receives even better support from the complete dissimilarity in the mobility curves of Cryptostegia latex particles and the isolated serum proteins. That there is a non-protein component of the particle surface is hardly to be doubted. I thought it very probable that this non-ionizable component is hydrocarbon, the same which constitutes the core of the latex globule.

Direct observation of incipient coagulation also revealed that there is no loss of identity of the globules and no destruction of the stabilization membrane on initial agglutination.

The foregoing experimental findings and the deductions based thereon met with some opposition. The surface of latex particles is generally assumed to be of pure protein, the hydrocarbon presumably not entering into the composition of the stabilization membrane at all.

The expression "stabilization membrane," as commonly used in the chemistry of emulsions, denotes all forms of coating on the surface of the globules, from monomolecular to multimolecular layers. Monolayers may occur in living systems, but multilayers cover cells and natural emulsions. The stabilization membranes of some artificial emulsions may actually be isolated as delicate pellicles. Monolayers receive emphasis because they are more amenable to physical-chemical laws.

When latex particles collide, one of two events could take place, the particle may coalesce, as when

two unprotected oil globules come into contact, or they may adhere and retain their identity. The former event will occur in a stabilized emulsion if, as a result of contact, the protective membranes break down. Through destruction of the membrane the hydrocarbon in a latex globule will be liberated and can then take part in coagulation, together with the protein constituents of the globule surface. If, however, the globules do not lose their identity and the original surface remains intact, then, when the globules collide and adhere, their stabilization membranes are alone responsible for the elastic qualities of the aggregate.

The evidence generally cited as proof that the stabilization membrane of Hevea latex is of protein and is destroyed on coagulation, is the fact that latex film is irreversibly coagulated by dehydration, presumably because the protein is no longer an effective stabilizing agent. This is not very convincing proof. Isoelectric points established by electrophoretic studies[1] give better support to a protein covering on latex particles, for the isoelectric points are protein precipitation maxima. But these studies also give evidence of a non-protein, non-ionizable component in the surface layer of latex globules. I was reasonably content with this evidence, but as the research done in Haiti dealt primarily with electrophoretic behavior and the composition of the surface but not with coagulation *per se,* and not with the fate of the membrane, it seemed advisable to verify my interpretation of coagulation. Although there is no visible destruction of the stabilization membrane on coagulation and therefore no evidence that hydrocarbon takes part in coagulation, release of the hydrocarbon nevertheless is attributed to a breakdown of the surface. Doubting this, I again journeyed into the field, to the Government Agricultural Station at Coconut Grove, Florida, where the director, Mr. Harold Loomis, placed the facilities of the station at my disposal, for which I here express my appreciation. Latices of the following plants were examined: *Cryptostegia grandiflora, C. magagascariensis,* the natural F_1 hybrid of the two foregoing species, *Ficus elastica, Hevea braziliensis, Manihot glaziovii, Funtumia elastica* and *Landolphia droogmansiana.*

The latices of the foregoing eight species of plants showed but slight differences in behavior, principally in isoelectric points. The general conclusions on coagulation are, therefore, applicable to all the species. Differences in behavior were often greater between the twig and trunk latex of the same plant than between different species. I shall, however, restrict my statements to two plants, Hevea and Cryptostegia.

The latices were placed in sodium acetate acetic acid buffers, the pH ranging from 2 to 7. Isoelectric points of the different species varied between pH 3.5 and 4.6.

The first step in the coagulation of latex is the coming together of two globules. On further agglutination there is evidence of polarity for the globules tend to join up in chains (Fig. 1a). Chain formation is greatly increased by stretching. The fibrous appearance of crude rubber in the coagulating vat is a microscopic picture of aggregates of the minute chains to be seen through the microscope.

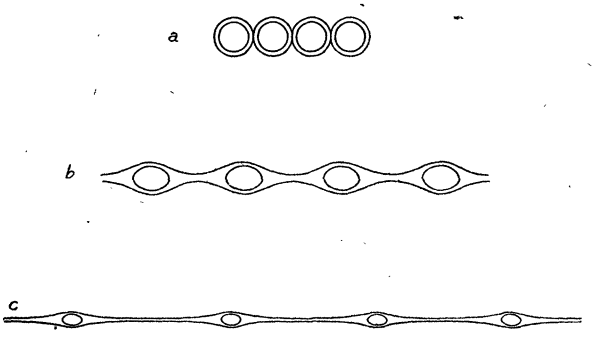

FIG. 1

Several microscopic fibrils or chains of agglutinated globules when grouped in parallel alignment form a microscopic aggregate which can be stretched by hand with the aid of needles. A single microscopic chain may also be stretched by a simple device. When a preparation containing latex at the isoelectric point is so mounted that numerous air bubbles are included, a single chain of latex globules may be found with one end attached to the surface of the glass slide and the other end held by tension to the surface of an air bubble. If now, the air bubble, by slight pressure is made to move to the edge of the slide, the chain of globules, which is actually a delicate strand of rubber, will be stretched. This picture, when viewed under a magnification of 1350x, reveals very clearly that it is the surface layer, the stabilization membrane, which is being stretched (Fig. 1b). Moderate stretching leaves the globules still intact, with no destruction of the membrane. Repeated stretching, done on larger threads which are fascicles of single chains, reduces the size of the globules through incorporation of more and more hydrocarbon from the core of the globule into the surface complex (Fig. 1c). Elasticity, that is to say, the degree of extensibility and rapidity of return, is very great. The globules, which are at first in direct contact, become more and more separated through repeated stretching (Fig. 1, a, b, and c). The intervening substance composing the much stretched thread is crude rubber; it is the former surface layer of the globules which has increased in bulk at the expense of the inner hydrocarbon.

The original membrane, which on initial contact

[1] *India Rubber World,* Aug. and Sept., 1945.

suffered no modification, has now, through repeated stretching, been so greatly modified that it has lost its identity as a surface covering (Fig. 1c). In order to escape the rather incongruous situation of switching from a fiber which is initially elastic because of the protein composition of its surface, to a later stage in the transformation of that fiber which is now elastic because of a protein-hydrocarbon complex, it is only necessary to assume that hydrocarbon enters into the composition of the original membrane. The feeble zeta potential on Cryptostegia latex globules, and the dissimilarity between the migration curves of latex globules and serum proteins, give experimental support to this view.

The foregoing deduction is in keeping with much work on the chemical nature of the surfaces of living cells and natural emulsions. Biologists have long regarded cells as coated with fatty substances. Recently, the milk globule, heretofore thought to be stabilized by casein, has been shown by Palmer[2] and by Moyer[3] to be a complex of phospholipids and protein. Moyer's work on milk is very similar, both as to method and the conclusion reached, to the study on latex reported here.

It is difficult to escape the conclusion that molecular continuity exists between the core and the surface of latex globules, and that the elasticity of crude rubber when first formed resides in a surface layer which is a composite of protein and hydrocarbon.

WILLIAM SEIFRIZ
UNIVERSITY OF PENNSYLVANIA

SCIENTIFIC APPARATUS AND LABORATORY METHODS

SEDIMENTATION OF POLIOMYELITIS VIRUS BY CENTRIFUGATION[1]

NUMEROUS reports have appeared in the literature showing that the virus of poliomyelitis can be sedimented by ultracentrifugation. The original report of Schultz and Raffel,[2] who effected partial purification from infected monkey spinal cord, has been amply confirmed, both for human virus and for the spontaneous mouse poliomyelitis of Theiler.[3,4,5,6] The method has been adapted for the recovery of virus from stools by Melnick,[7] and more recently the same author has demonstrated the presence of virus in the blood of experimentally infected monkeys by this technic.[8]

In all these reports, air-driven ultracentrifuges have been used, running at speeds of 22,000 to 39,000 r.p.m. for periods of one to six hours. It occurred to us that prolonged centrifugation at lower speeds, in an apparatus more readily obtainable and less expensive, might be effective in sedimenting virus.

For this purpose, experiments have been performed using the International Equipment Company Multispeed Attachment on an ordinary Size 2 centrifuge. The head of this attachment, six inches in diameter, holds six tubes at a 45° angle, with a total capacity of 33 cc. Speeds up to 18,000 r.p.m. are obtainable, at which a force 25,000 times gravity is exerted.

EXPERIMENTAL

For all experiments the mouse-adapted Lansing strain of poliomyelitis virus was used. A 20 per cent. stock suspension was prepared in saline and centrifuged at 18,000 r.p.m. for 20 minutes, the clear supernate being stored in ampoules on dry ice. On thawing, some particulate matter appeared which was removed by re-centrifugation at 18,000 r.p.m. for 10 minutes before the experimental tests. For the latter, a period of four hours at 18,000 r.p.m. was arbitrarily selected. Shorter periods were not employed. To keep the centrifuge head below room temperature, the centrifuge chamber was cooled by the addition of about five pounds of dry ice. Centrifugation was interrupted hourly to replenish the supply. Experiments were performed with 20 per cent. and with 1 per cent. suspensions, both in saline.

With 20 per cent. virus a small, transparent, amber-colored pellet was formed. With 1 per cent. virus, a film was deposited over the lower surface of the tube. After centrifugation, the tubes were washed twice with saline, without disturbing the sediment. The gummy sediment was then triturated with a glass rod in a drop of saline, and diluted with saline to the original volume. Resolution was not complete, and in the case of 20 per cent. virus the reconstituted sediment was centrifuged at low speed for a few minutes before diluting it for titration, a certain amount of virus presumably being lost. Mice were inoculated intracerebrally with 0.03 cc of the clear supernate.

The results of four experiments are summarized in Table 1. In the first, the top and bottom thirds of

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

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[7] J. L. Melnick, *Jour. Exp. Med.*, 77: 195, 1943.

[8] J. L. Melnick, *Proc. Soc. Exp. Biol. and Med.*, 58: 14, 1945.

[2] *Jour. Biol. Chem.*, 104: 359, 1934.

[3] *Jour. Biol. Chem.*, 133: 29, 1940.