Latex Flow Studies III. Electrostatic Considerations in the Colloidal Stability of Fresh Hevea Latex

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Fresh Hevea *latex is a dual colloid system. As a first approximation it consists of negatively charged particles of various types suspended in a serum containing anionic proteins. Included in the negatively charged suspended particles are membrane-bounded bodies (lutoids) which enclose a second colloid system in which particles are suspended in an acid, and predominantly cationic, serum. Destabilisation occurs if the two systems come in contact. Electron microscopy throws some light on the details of the destabilisation. Zeta potentials and surfactant titration methods have been investigated as a means of estimating serum effectiveness. Enzyme destabilisation may follow an initial and faster electrostatic destabilisation.*

Fresh latex from *Hevea brasiliensis* is a colloid system of extraordinary complexity and interest. It has been shown to be a dilute whole cytoplasm. Whereas cytoplasm of any kind is normally rather difficult to obtain except in small amounts, *Hevea* latex is regularly available in very large quantities in the natural rubber producing countries. It thus offers unusual opportunities for study. Freshly collected *Hevea* latex is a polydisperse system containing a wide range of suspended bodies in an ambient serum referred to in the literature as C-serum (abbreviation of 'centrifuge serum' since it can be separated by high speed centrifugation). Latex as sold commercially is a concentrated suspension of rubber hydrocarbon from which a large proportion of the original non-rubber components has been removed (usually by centrifugation) and to which preservatives, such as ammonia, have been added. The great majority of colloid studies of latex have been directed at this simplified system, the fresh latex considered in this paper being of a different order of complexity. It is still possessed of active organelles and enzyme systems and, for a time, is able to carry out biosynthetic reactions. It is a fertile medium for bacteria and as normally collected is always infected, usually carrying a population of 10^5 to 10^6 bacteria per ml (TAYSUM, 1958). Because of bacterial infection and normal degradative changes when removed from its natural environment, fresh latex 'dies' within a few hours, and as the bacterial population rises, it becomes acid-sour and curdles. These processes can be slowed down by special techniques of collection and by handling at low temperatures.

Colloidally the dominant particulate phase of freshly collected latex is rubber hydrocarbon. Particles of this make up 30–45% of the whole volume and occur in a particle size range of 3 μ to 0.02 μ with the majority in the region of 0.1 μ. Next in abundance are the 'lutoid' particles amounting to 10–20% of the volume. Many other types of particle are present, but all in minor amounts. The lutoids are subcellular membrane bounded bodies, visible under phase contrast optics, and typically 2–5 μ in diameter. Their membrane encloses a fluid serum in which particulate bodies are often dispersed. These particulate inclusions are small and in vigorous Brownian motion. The lutoid thus constitutes an enclosed colloid system in its own right. After collection, lutoids usually have rather a short life. Their entrapped particles come out of suspension and stick to the membrane, which appears to thicken and then breaks, discharging the interior serum into the latex as a whole. Lutoids are relatively heavy and can be separated by centrifugation. They can be resuspended in 0.4 M

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mannitol solution (lutoids are osmotically sensitive) for study. Their membranes can be ruptured by repeated freezing and thawing (HSIA, 1958) or by ultrasonics (RUBBER RESEARCH INSTITUTE OF MALAYA, 1961) so that the interior serum can be collected. This lutoid serum (often referred to as B-serum because it was first obtained by HSIA from 'bottom' centrifuge fractions) has quite different properties from the ambient C-serum in which the intact lutoids, with other particles are originally dispersed. B-serum, as described by HSIA (1958), was prepared by treatment of all the sedimentable fractions from latex. It has been shown that lutoids, after separation by repeated centrifugation and washing, yield a serum indistinguishable from B-serum as regards protein pattern on starch gel electrophoresis (SOUTHORN AND EDWIN, 1968), the inference being that sedimentable particles other than lutoids make very little contribution to it. B-serum contains proteins not found in C-serum (MOIR AND TATA, 1960; TATA AND MOIR, 1964; KARUNAKARAN *et al.*, 1961) and is rich in enzymes. Acid phosphatases, proteases, polyphenol oxidases, phospholipase D, and lysozyme have been reported (HSIA, 1958; PUJARNISCLE, 1965; AUDLEY, 1965; PUJARNISCLE AND RIBAILLIER, 1966; RUBBER RESEARCH INSTITUTE OF MALAYA, 1965 and 1966).

As a colloid, the lutoid is of interest in that its serum (B-serum) has been shown to be a destabiliser for rubber hydrocarbon particles (SOUTHORN AND EDWIN, 1968); but, C-serum can, to some extent, protect against this destabilisation. Thus the colloid stability of fresh latex is greatly influenced by a balance of effect between the C-serum and such B-serum as has been released at any given time.

This investigation was undertaken to obtain information on the mechanisms of this interaction. It is already known that the rubber hydrocarbon particles of latex owe their colloidal stability to a negatively charged protective envelope of hydrophilic colloids; the envelope including protein and phospholipid components. It has been suspected that the lutoid B-serum contains enzymes (phospholipase and protease) capable of attacking this envelope (RUBBER RESEARCH INSTITUTE OF MALAYA, 1965 and 1966). The authors at first thought that this might be a sufficient explanation, but the present work suggests that the postulate of enzyme attack, though possibly real, only provides a partial explanation.

OBSERVATIONS

One of the difficulties in the theory of enzyme attack lies in the kinetics of the destabilisation and the form of the precipitated rubber. Lutoid serum will destabilise rubber suspensions very rapidly, while attempts to duplicate this activity with activated protease and phospholipase systems suggest that an appreciable reaction time is required. Furthermore lutoid serum precipitates the rubber initially in the form of a cream of small flocs, whereas enzymes alone usually produce an irreversible rubber gel or coagulum which then undergoes syneresis (EDWIN, 1965).

If destabilisation were entirely due to natural enzymes one would expect that their action might be specific to surfaces having a protein/phospholipid envelope of the type characteristic of the natural rubber hydrocarbon particle. The following preliminary experiments were designed to check this suggestion.

Lutoid serum was prepared by first separating lutoids from freshly collected latex, washing the lutoids in isotonic sugar solution, breaking their membranes with alternate freezing and thawing, and removing the membrane residues by high speed centrifugation (SOUTHORN AND EDWIN, 1968). The lutoid serum was then mixed with suspensions of the following materials:

(a) Rubber hydrocarbon particles obtained by centrifugation of fresh latex, resuspended in water to a concentration of approximately 5% at pH 6.5.

(b) Rubber hydrocarbon particles obtained from commercial latex concentrate stabilised with ammonia and held in store for one year. The particles were further concentrated by high speed centrifugation and redispersed in distilled water to give a concentration of approximately 5% rubber at pH 6.5.

(c) A polystyrene latex [Dow Chemical Co., standard particle size latex (0.577 μ)]

diluted to approximately 5% with distilled water to pH 6.5.

(d) A suspension of quartz particles dispersed in water at pH 6.5. This dispersion was not completely stable but the quartz remained in suspension for several hours if left undisturbed.

On addition of each of the above suspensions (0.2 ml) to lutoid serum (1.5 ml), visible flocculation was observed in all cases within 30 minutes. It is evident that the particle surfaces are very different as between one suspension and another. With the ammonia stabilised concentrate, it may be assumed that after a year's storage in contact with ammonia, the original protective envelope of protein phospholipid will be mostly replaced by one of fatty acid soaps. The exact nature of the surface of the polystyrene particles is unknown but will certainly differ from that of rubber hydrocarbon in fresh latex. No stabilising colloids were added to the quartz suspension at all.

A stabilising feature which all these suspensions have in common is that in all cases the suspended particles carry a negative charge. It therefore seemed possible that electrostatic interactions played a part in the activity of the lutoid serum.

At this point the possibility was considered that lutoid serum might contain sufficient amounts of inorganic cations to account for this general destabilising activity. Analysis showed the presence of Na^+, K^+, Ca^{++} and Mg^{++}. The experiments were therefore repeated with dialysed serum. Lutoid serum was dialysed overnight in a 'Visking' membrane of approximately 24 Å pore size against continuously renewed distilled water. The experiments were repeated next morning, with identical results. The authors were therefore led to the conclusion that lutoid serum contains a cationic material of high molecular weight, probably protein, in addition to inorganic cations.

The protein constituents of lutoid serum (B-serum) and the ambient serum (C-serum) have already been examined by starch gel electrophoresis (MOIR AND TATA, 1960; TATA AND MOIR, 1964; KARUNAKARAN *et al.*, 1961). The typical starch gel pattern at pH 8.6 is shown in *Figure 1*. It is evident that the main protein constituents of C-serum are anionic, whereas the B-serum pattern shows a major contingent of cationic proteins. Since the starch gel experiments were done at rather high pH (pH 8.6), it is possible that lutoid serum (at pH 5.4) as prepared in the above experiments contains still further proteins on the cationic side of their isoelectric point.

Further experiments were designed to check whether cationic proteins could in fact reproduce the observed destabilising behaviour of lutoid serum. For this purpose, cytochrome-C (General Biochemicals Inc., Ohio) was chosen as a protein of high isoelectric point (p.i. 10.5) without lytic activity. Preliminary analysis showed that the protein content of lutoid serum is normally about 3%. Solutions of cytochrome-C in distilled water at concentrations of 1, 2, 3 and 4% were prepared and added to 2% suspensions of rubber hydrocarbon particles separated by centrifugation from fresh latex and resuspended in distilled water. In all cases, an immediate flocculation was observed. When lutoid serum is added to rubber suspended in its native C-serum rapid visible creaming of rubber is observed only when the ratio of lutoid serum to C-serum exceeds about 20%, due to the protective action of the C-serum. This experiment was repeated with solutions of cytochrome-C. In this case the 4% aqueous cytochrome-C solution induced visible creaming of rubber when added in concentrations relative to C-serum exceeding 20%, *i.e.*, a 4% cytochrome-C solution behaves in a remarkably similar fashion to lutoid serum which is believed to contain approximately 3% protein. The analogy is striking and indicates that a cationic protein could produce the effects observed with lutoid serum at the same order of concentration. The protective action of C-serum could readily be explained as an electrostatic effect since the starch gel pattern (*Figure 1*) shows that the C-serum proteins are anionic and would interact with the cationic material of the lutoid serum.

On this basis it is not surprising that a mixture of lutoid serum and C-serum in the absence

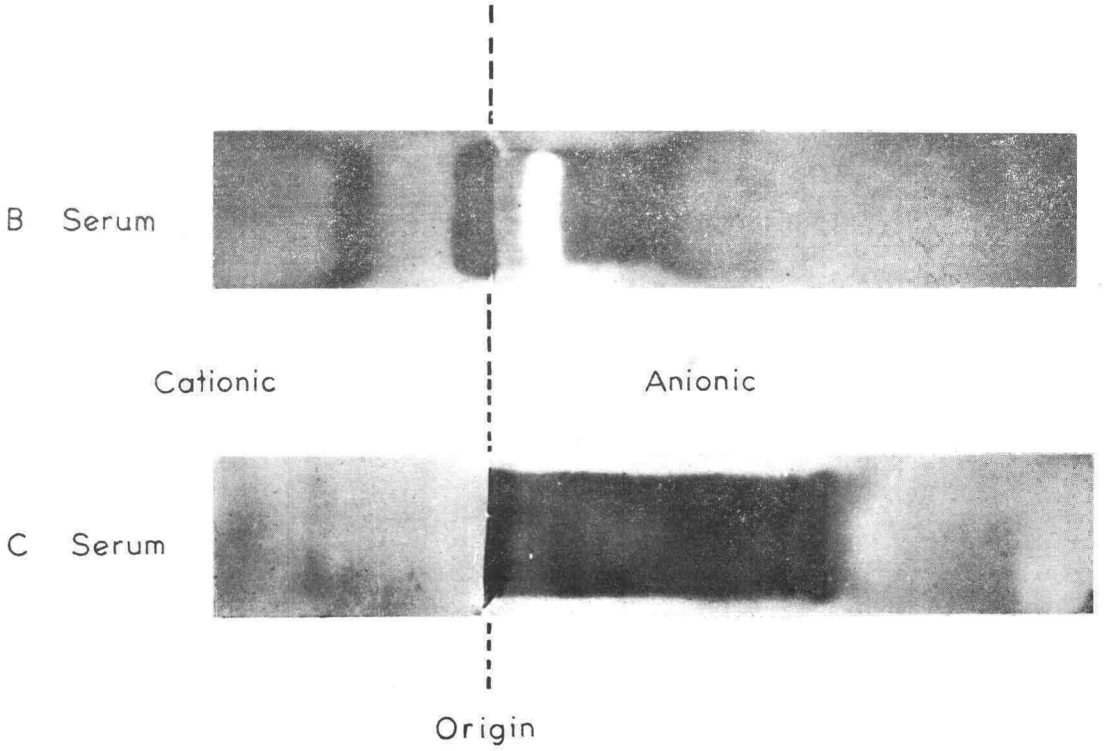

Figure 1. Starch gel electrophoresis pattern for B- and C-sera.

of rubber interacts and mutually precipitates (SOUTHORN AND EDWIN, 1968).

One would also expect that a dispersion of intact lutoids would aggregate if their own internal serum was added to the dispersing medium, for the outer surface of the intact lutoid should carry an external negative charge like any other particle dispersed in latex. It is a well known characteristic of lutoids that as they age, they show an increasing tendency to aggregate (HOMANS AND VAN GILS, 1948; SOUTHORN, 1961) but lutoids separated quickly by centrifugation from latex collected under good conditions can be dispersed in isotonic sugar solutions. An experiment was carried out in which latex was collected into chilled glass containers neglecting the first three minutes of flow (which usually has a high proportion of damaged lutoids). The lutoids were separated from the latex by centrifugation and redispersed with addition of a small amount of 0.4 M mannitol solution in water, using a plunger-type homogeniser. A fairly good dispersion was obtained, though examination under the microscope showed some lutoids aggregated in small groups of two or three lutoids per group (it is rarely possible to get a better dispersion than this after centrifugation). The dispersion was added to an equal volume of B-serum. All the lutoids clumped together and could not be redispersed, though examination under the microscope showed them still as intact particles. Thus the tendency for lutoids to aggregate on standing is probably linked with release of B-serum. Apart from the effect of a general release of B-serum by lutoid breakage, in the case of a lutoid even a very small amount of cation leakage across its membrane, would reduce external charge and destroy the electrostatic component of stability for the

lutoid concerned. This could happen without membrane rupture, and is probably the reason why lutoids are always the first particles to aggregate as latex ages; this occurs well before the lutoids actually break down.

The destabilising action of cationic proteins suggests that cationic surfactants added to latex should exert a destabilising effect. This is in fact well known. Cetyl pyridinium chloride (CPC) is a very powerful destabiliser at low concentrations of surfactant [at high concentrations it is capable of reversing the charge on the rubber particle giving rise to a stable dispersion of positively charged rubber (BLOW, 1938)]. It is worth noting that CPC destabilises fresh latex in such a way as to produce rapid creaming of rubber floccules, the appearance of the destabilised system being similar to that of latex destabilised by lutoid serum.

Attempts were then made to put these findings on a more quantitative basis. If lutoid serum shows cationic surface activity, it should be possible to estimate this activity by titration against an anionic surfactant of known composition. Similarly, if the protective action of C-serum is partly due to anionic surface activity, then C-serum should be titratable against a cationic surfactant.

Several considerations were taken into account in choosing suitable surfactants: they should be available in a state of high purity, they should be of definite known composition, and their solutions in water should be compatible as regards pH with the serum to be titrated. Both lutoid serum and C-serum exert a buffering action towards their characteristic pH values, 5.4 in the case of lutoid serum and 6.9 in the case of C-serum. The authors were reluctant to add additional buffers since the two sera are already complex systems and they did not wish to increase this complexity. Surfactants were therefore chosen which gave dilute aqueous solutions of pH not far removed from the natural pH of the serum to be titrated.

As a cationic reagent, CPC was found very suitable, being available in pure crystalline form. Sodium dioctylsulphosuccinate (Aerosol OT) was also found to be a suitable anionic surfactant: this in a 100% concentration is a white curd soluble in water.

There are abundant references in literature to surfactant titrations, usually employing a dyestuff such as pinacyanol blue to make the end-point more readily visible. In the present work the determination of end-point presented a difficulty in that both sera are slightly turbid and the lutoid serum, because of its polyphenol oxidase content, undergoes variable colour changes after preparation. The authors tried carrying out the preparative procedures for B-serum under nitrogen and sweeping out the reaction tube during titration with a stream of nitrogen, but it was difficult to avoid the darkening effect entirely. The increase of turbidity at the end-point appeared to offer good prospects, and a simple titration apparatus based on light-scatter measurement (*Figure 2*) was constructed. An improved model, now being made, will be described in a later publication. In the version used for these experiments, the reaction vessel was held in a duralumin block underneath which was a magnetic stirrer. A photo-electric cell received light through the reaction mixture. Two light sources were provided so that either direct transmission or scatter at 90° angle could be measured. The transmission facility was used for calibration only. In use, the surfactant was placed in the reaction vessel with a magnetic 'policeman'. The serum to be titrated was added to the stirred surfactant at constant rate from a motor driven syringe burette. The photo-cell current from 90° scatter illumination was taken through a bridge circuit to an amplifier, and the results were plotted on a chart recorder.

It was found possible to carry out reproducible titrations with this device. *Figure 3* shows a duplicate experiment with CPC titrated against Aerosol OT. There was a sharp peak of turbidity when the molecular concentrations of the two reactions reached parity. In this condition the cations and anions were presumably neutralised, micelle formation was inhibited, and solubility of the complex was reduced. As more of one reactant was added, complex micelle formation was possible and the initial precipitate was solubilised so that light scatter dis-

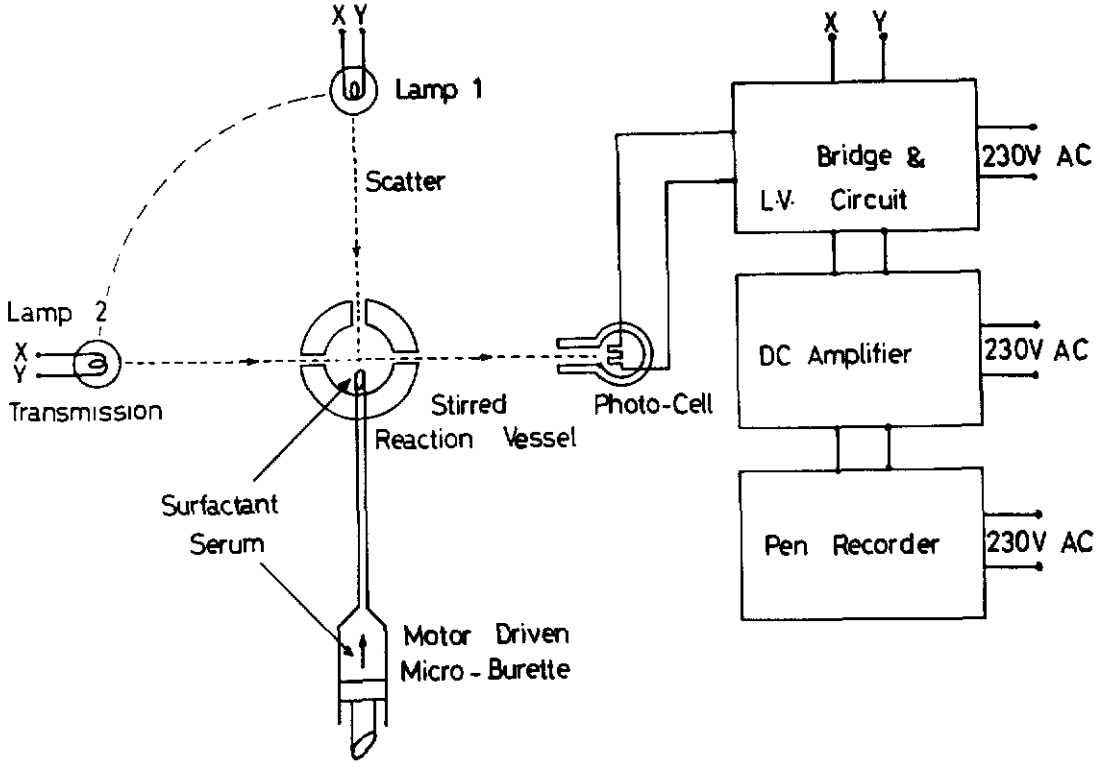

Figure 2. Schematic diagram of apparatus for titration on a charge basis using 90° light scatter to detect a turbidity end-point. Standard surfactant solution is placed in the reaction vessel, serum in the motor-driven syringe micro-burette.

appeared. Titrations of reactants such as CPC against Aerosol OT could be carried out satisfactorily in either direction (*i.e.*, with either reactant in the receiver).

In the case of titrations of surfactant against a protein the conditions are rather different. If the surfactant was added to the oppositely charged serum, protein was immediately thrown out of solution because excess of protein was incapable of solubilising the precipitate. Light scatter therefore increased as more surfactant was added until substantially all the protein was precipitated. Further addition of surfactant decreased the scatter slightly, partly by its dilution effect and partly by solubilisation in the presence of excess surfactant. Variation of the serum concentration made relatively little difference to the initial shape of the curve though of course the intensity of maximum scatter was altered. A more favourable condition for titration was to add the serum to the surfactant. In this case the initial precipitate was solubilised by excess of surfactant and scatter did not become significant until the surfactant began to be used up. Scatter then increased rapidly to a peak, after which further addition of serum produced only a slight diminution of scatter because of dilution. For these reasons the surfactant was always placed in the receiver. Titration curves for Aerosol OT against lutoid serum are shown in *Figure 4*. Similar curves for C-serum are shown in *Figure 5*. The waver in the chart lines is due to inadequacies in the transmission of the

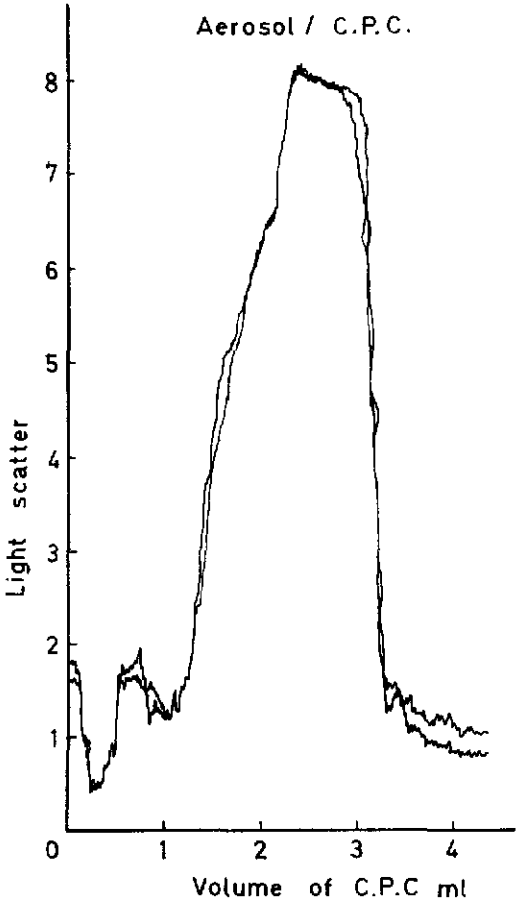

Figure 3. Duplicate titration of Aerosol/CPC Receiver contains 1 ml 0.005M Aerosol, CPC (0.004M) solution is added from syringe micro-burette.

signal to the recorder which the authors hope to remove in the later model now being constructed. It will be noted that minor scatter peaks occur near the origin which may be due to the complex nature of the sera. The main interest in these experiments at present lies in the fact that it is possible to titrate lutoid (B-) and C-sera against surfactants of opposite charge as was postulated, and thus to arrive at a numerical estimate of their potency on a basis of charge neutralisation.

These techniques were used to examine the 'potency' of B- and C-sera separated from a number of collections of latex in terms of their charge effects when titrated against surfactants. Some results are shown below in m/equivalents of standard surfactant. *Table 1* gives an idea of the reproducibility of the measurement. For this experiment, in order to get a sufficient amount of material for repeat titrations, B- and C-sera were frozen and pooled from collections over a period. The measured activities of the pooled, stored sera are somewhat lower than those of the fresh sera as shown in *Table 2* in which day-to-day titration results are shown for B- and C-sera from latex of three clones. It would seem that the sera lose activity on storage even when frozen. In *Table 2* the serum activity is also shown as a ratio of percentage B-serum activity in relation to C-serum activity from the same latex. This is of interest in that the stability of the latex will depend in part on a balance of cationic to anionic activity.

It is evident that wide day-to-day and source

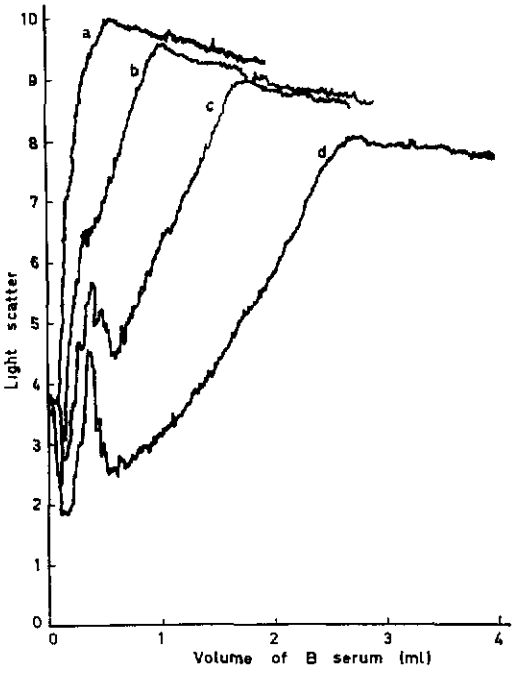

Figure 4. Serum titration against surfactant. B-serum at various dilutions with water placed in burette, Aerosol OT (0.006M) in receiver (1 ml). a—100%, b—50%, c—25%, d—12.5%.

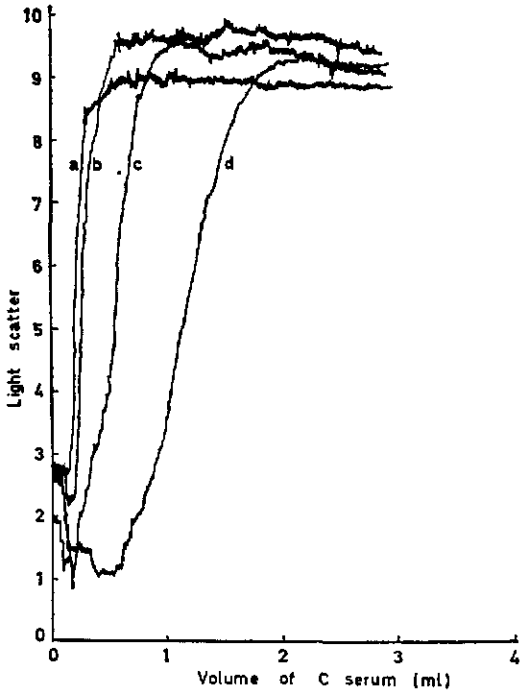

Figure 5. Serum titration against surfactant. C-serum at various dilutions with water placed in burette, CPC (0.004M) in receiver (1 ml). a—100%, b—50%, c—25%, d—12.5%.

variations occur in 'potency', especially for cationic (+ve) activity of B-serum.

From these results, the idea that lutoid serum can destabilise rubber by charge neutralisation seemed fairly well established. In view of the importance of this finding to future work, further confirmation was sought by attempting to determine the zeta potential of rubber hydrocarbon particles from fresh latex and to see how this would be modified by addition of lutoid serum.

The method adopted was that of direct observation in a microcatephoresis cell. The rubber hydrocarbon particles of fresh latex are extremely small and in rapid Brownian motion, to observe them individually in a defined plane requires high optical magnification, preferably with oil immersion objectives. In this case the available working distance must be very small and the cell must be made very thin, especially since it is desirable to check cell performance by determining velocity gradients over the complete cell depth. For this purpose a very simple cell was devised. To a normal $2\frac{1}{2}'' \times 1''$ microscope slide, two squares of copper foil were cemented with Araldite as shown in *Figure 6*. The foils were bent as shown to form convenient sockets for the leads to the power source. A number of such slides was prepared. After thorough cleaning, the slides were placed in an Edwards Model H vacuum coating apparatus, and a conducting film of gold was evaporated onto them. Before placing in the dome, each slide was provided with a paper masking strip 0.5 cm wide placed across the centre of the slide. Removal of the strip after depositing the gold film left an uncoated area 0.5 cm wide across the glass. The copper plates were used only to provide a robust contact to the gold film. In use a mixture of vaseline/mineral turpentine of suitable consistency for painting with a brush was made up. Using this mixture a square of cover glass dimensions was painted (with number 00 camel hair brush) symmetrically in fine lines bridging the gold/glass/gold gap. In the centre of this square a measured drop of suspension was placed. A cover glass was then lowered gently onto the drop which then filled the vaselined square. Cells of this type had excellent optical properties, and the authors found it possible to scan

TABLE 1. REPRODUCIBILITY OF SERUM TITRATIONS AGAINST STANDARD SURFACTANTS

Titration repeated	+ve Activity of B-serum (against Aerosol OT) $\times 10^{-3}$	−ve Activity of C-serum (against CPC) $\times 10^{-3}$
1	7.63	7.38
2	7.85	7.38
3	7.63	7.38
4	7.63	7.82
5	7.85	7.38

TABLE 2. DAY-TO-DAY TITRATION RESULTS OF ACTIVITIES OF B- AND C- SERA PREPARED FROM THE SAME LATEX FOR DIFFERENT COLLECTIONS, FROM THREE CLONES

Clone	+ve Activity of B-serum (against Aerosol OT) $\times 10^{-3}$	−ve Activity of C-serum (against CPC) $\times 10^{-3}$	% Activity of B-serum in respect to C-serum of the same latex
RRIM 526	10.0	11.0	91
	6.6	9.8	67
	8.9	12.7	70
	8.9	10.4	86
	2.5	10.5	119
	8.0	13.7	58
PR 107	9.6	11.5	84
	8.7	10.9	80
	8.9	11.5	77
	10.4	10.3	101
	7.8	13.2	59
	7.6	12.3	62
Tjir 1 (Tree A)	14.2	12.8	119
	13.2	13.2	100
	13.7	12.8	107
	15.4	12.8	120
	11.0	12.8	86
	10.4	12.8	81
	13.7	13.2	104
	13.7	13.7	100
	12.8	13.2	97
	11.3	13.2	86
Tjir 1 (Tree B)	11.0	12.8	76
	13.7	12.8	107
	11.3	10.9	117
	12.8	11.0	116
	9.2	7.5	123
	11.5	9.6	114
	15.4	13.2	117
	8.1	13.2	62
	14.2	10.7	133
	10.2	9.6	106

right through their depth using oil immersion phase contrast optics. Dimensionally the cell proved surprisingly reproducible and stable. Normally such a cell was used for one experiment only, after which it was cleaned of gold and recoated. Thus, in a series of experiments, many such cells would be used in much the same way as a microscopist uses a different slide for each preparation. The cell thickness was measured before each measurement and at the end of each experiment. After a little practice the variation of cell depth between slides became very small and movement of the cover glass (which would affect cell thickness and velocity gradient) did not in fact occur. The electrodes are of course the edges of the gold coating, and in such a system there is an obvious risk of electrode effects including

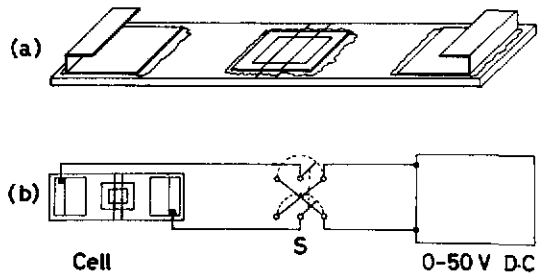

Figure 6. (a) Sketch of microcatephoresis cell consisting of copper-foil contacts cemented to a microscope slide which is then gold-plated save for a transverse strip in the middle. This strip is ringed with paraffin and bridged by a cover slip forming the cell proper. (b) Electrical arrangements for cell. S is a polarity reversing switch.

polarisation. A variable voltage power pack (LKB 0–50 V transistorised power supply) was used. As an initial check the motion of an individual rubber particle at 50% of the cell depth was measured at increasing voltage gradient with results as shown in *Figure 7*. These results indicate a reasonable linearity, in practice voltage gradients in the range A–B of *Figure 7* were used. The higher voltages of this range were used only where mobilities were so low that the observation was otherwise unduly prolonged, with the attendant risk that the particle would disappear from the sharp focal plane of the oil immersion objective. Particle movements were timed across a calibrated eye-piece graticule, and were for periods of a few seconds only, being made first with one polarity and then with reversed polarity. The values taken are the means of several such measurements for each condition. *Figure 8* shows the distribution of particle velocities across the cell for a fixed potential gradient. For estimation of zeta potential the measurements were all made at 21.2% cell depth to minimise errors due to counter-current motion (SMOLUCHOWSKI quoted by ABRAMSON, 1934). Because the results for any cell depth show a certain scatter, the authors took mean velocity measurements at different depths scanning through the cell, and then drew the best velocity gradient possible, taking the estimated mobility as the mean of the two intercepts at 21.2% depth (*Figure 8*). No great claims for absolute accuracy can be made for such a simple apparatus.

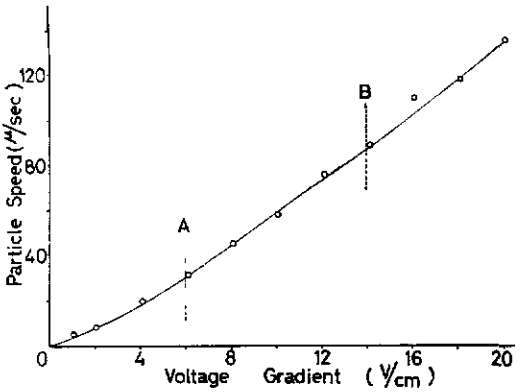

Figure 7. Particle migration rates at mid-cell for increasing voltage-gradient. In practice gradients between the limits A and B were used.

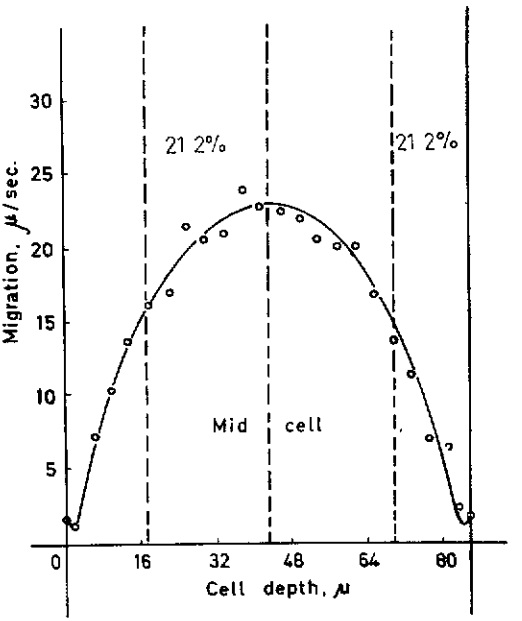

Figure 8. Particle migration rates through cell from cover slip to slide.

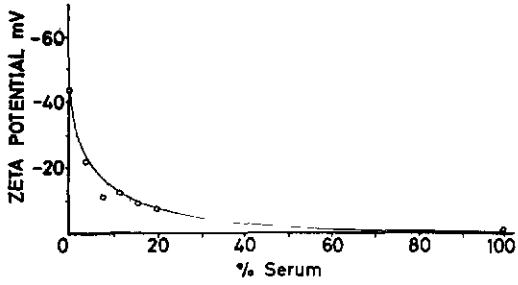

Figure 9. Effect of adding B-serum to a suspension of rubber particles.

The experimental results are of course mobilities (μ). Zeta potential (ζ the charge at the shear interface) is related to mobility by the equation $\zeta = K\mu$ where $K = 4\pi\eta/\tau$ developed independently by SMOLUCHOWSKI (1903) AND PERRIN (1904) from an earlier treatment by HELMHOLTZ in 1879 (FREUNDLICH, 1926). The conversion of mobilities to zeta potential thus requires an estimate of dielectric constant τ and viscosity η under the appropriate boundary conditions. This can be a matter of some difficulty and debate, so that many workers prefer to avoid the conversion. In this case the authors have expressed the results as zeta potentials assuming a dielectric constant of 80 and a viscosity of 1 centipoise taking the view that no likely errors in the absolute values of ζ affect the conclusions drawn and that the arguments are easier to follow if discussed in terms of electrical charge. On this basis the zeta potential of rubber hydrocarbon particles in fresh latex ranged for different preparations from -35 mV to -45 mV, which is in reasonable agreement with published estimates from other workers (HAUSER AND BENDER, 1938; BOWLER, 1953).

Figure 9 shows the effect of adding lutoid serum to a suspension of rubber particles from fresh latex separated by centrifugation as 'white fraction' and resuspended in distilled water at a 2% rubber dispersion. The procedure was to make up mixtures of B-serum and water in graded proportions from zero to 100% B-serum. 0.8 ml of 2% rubber dispersion was added to 2.88 ml of the mixture, these figures being convenient for the automatic dispenser used to ensure rapid preparation. Thus the final rubber content was 0.44% and the highest concentration of B-serum 78%. The percentages on axis of *Figure 9* refer to the B-serum concentrations of the 2.88 ml mix before adding to 0.8 ml of suspension. A similar experiment was carried out using dialysed B-serum (*Figure 10*) the dialysis being carried out against continuously replaced distilled water, with the B-serum in a bag of 'Visking' membrane (estimated pore size 24 Å.) The process of dialysis results in some dilution of the B-serum so that the nominal B-serum concentrations shown in *Figures 9* and *10* are not precisely comparable. Despite these complications the two experiments show that B-serum, whether dialysed or not (*i.e.*, irrespective of whether small inorganic cations have been removed) reduced the negative charge on the rubber particle (ζ potential) when added to the dispersion. The reduction in ζ potential was rapid at first but tailed off later as increasing amounts of B-serum were added. In the case of undialysed B-serum, it was possible to reduce ζ potential to near zero, dialysed B-serum was rather less effective even allowing for dilution arising from the dialysis, presumably because the inorganic cations removed by dialysis were no longer able to contribute their share of activity.

It is interesting to compare the effects of lutoid serum on rubber particles with that of a surfactant such as CPC. *Figure 11* shows an experiment on the same lines, in which CPC

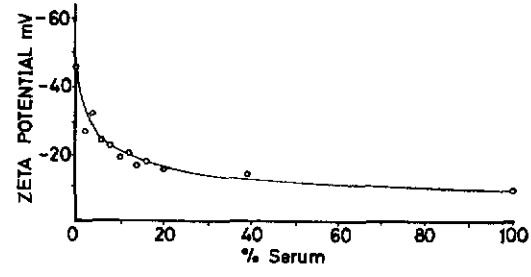

Figure 10. Effect of adding dialysed B-serum to a suspension of rubber particles in water.

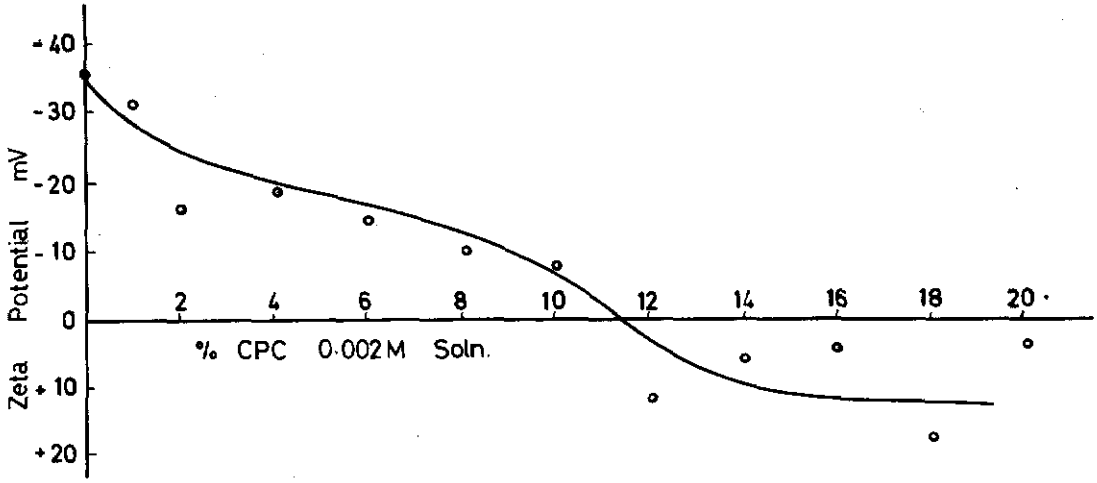

Figure 11. Effect of adding CPC to a suspension of rubber particles in water.

was added in various concentrations to rubber hydrocarbon (from fresh latex) suspensions. (As before 2.88 ml of CPC/water mixtures in graded concentrations was added to 0.8 ml of 2% rubber suspension). Whereas the lutoid serum components are apparently bound to the rubber by electrostatic forces only and therefore cannot do more than reduce the charge to a little above zero, CPC is adsorbed much more strongly by forces such that the initial envelope is displaced and the charge reversed. Similar results have been found for rubber hydrocarbon suspensions from preserved latex (BLOW, 1938), it is not too surprising that fresh latex rubber behaves in the same way, the results are mainly of interest as confirmation of the validity of our experimental methods.

These experiments confirm the previous indications that lutoid serum (B-serum) can destabilise rubber hydrocarbon particles by electrostatic interaction. B-serum reduces the negative charge of the particle to near zero if enough is added. The cationic components of B-serum whereby this is achieved range from small inorganic cations such as Ca^{++} to large molecules, almost certainly proteins. In practice it is not usually necessary to eliminate the charge on the particle completely in order to cause colloidal instability, consideration of the exact ζ potential level needed to bring this about would have to take into account many other factors. In the present case, where cationic proteins would seem to be involved, one of these many factors is the configuration and dimensions of the proteins concerned. It is easy to conceive of a monolayer of small surface-active molecules around a rubber particle but in the case of the smallest rubber particles we could well be dealing with interactions involving proteins of linear dimensions much exceeding the particle diameter. In this case destabilisation could well occur initially by formation of protein bridges between particles. This speculation is of interest in that if correct it might be possible to visualise the proteins concerned by electron microscopy. For this purpose very high magnifications are required and it was decided to work with only the smallest rubber particles, partly to make identification of protein bridges easier should they occur, and partly from the practical consideration that a large particle (say 1 μ diameter) would fill the entire field of view at the magnification needed.

Fresh latex was fractionated by repeated centrifugation and redispersion in water so as to collect a sample of very small rubber particles. A dilute aqueous dispersion of these was

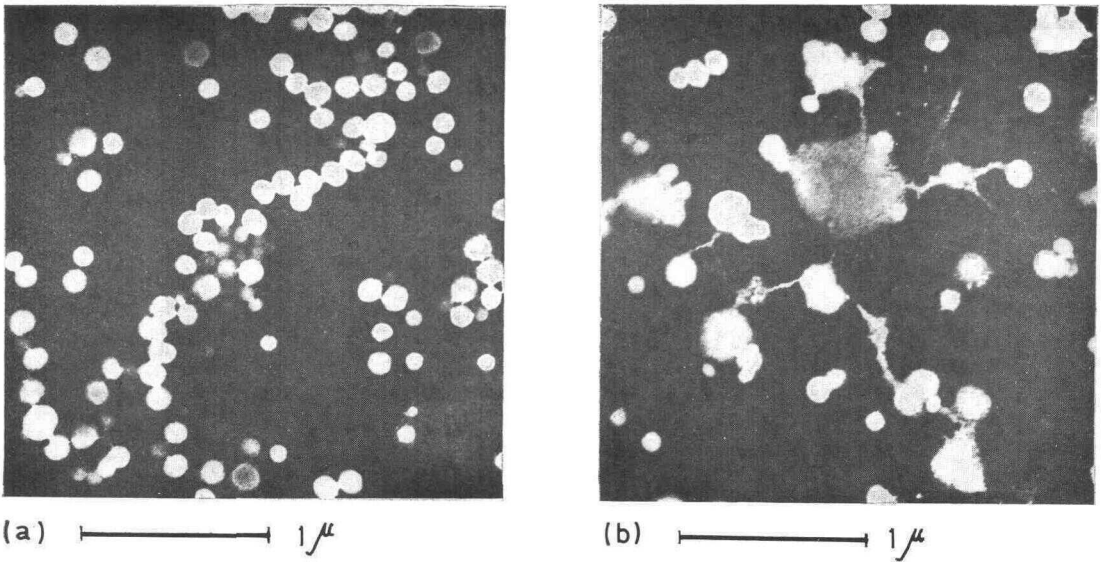

Figure 12. Electron micrographs of small rubber particles in (a) distilled water (b) B-serum.

prepared. A PTA fixed whole mount preparation of these is shown in *Figure 12 (a)* (Philips E.M. 100 electron microscope examination at 60 kV). Two drops of the original unfixed dilute rubber dispersion was then added to 5 ml dialysed B-serum with stirring. After 10 minutes a drop of the mixture was added to 1 ml of 1% phospho-tungstic acid (PTA) and one drop of the PTA/rubber/B-serum mixture dried down on a carbon coated grid for examination at 60 kV as before. The effect of the above procedure was to give a negatively stained image, which is often very effective for revealing protein structures. PTA is not an effective fixative for rubber however, so the rubber particles tend to spread and give rather blurred images. The results are given in *Figure 12 (b)* which shows rubber particles linked together by what appear to be long bridges (up to 0.5 μ) of protein. There is a suggestion of a periodic structure in the protein, which may be helical.

These pictures suggest there is at least one protein in B-serum which is capable of destabilising rubber by forming bridges between particles. It would be most unwise to extrapolate this result to the assumption that all the destabilisation phenomena in which B-serum is implicated relate to the particular protein, or even that the formation of long protein bridges is the only mechanism involved. Rather similar bridging mechanisms have been reported for other colloid systems by RIES AND MEYERS (1968) who note that charge neutralisation and bridging may occur simultaneously.

DISCUSSION

The observations show that fresh latex must be considered as at least a dual colloid system. Large numbers of particles of various types are dispersed in an ambient serum (C-serum) which contains proteins of anionic character at normal latex pH. As might be expected all these particles carry a negative charge, in the case of the rubber hydrocarbon particles the zeta potential has been measured and is of the order of −40 millivolts. However within this not unusual type of dispersion are membrane-bounded bodies (lutoids) which, though they would initially carry a negative charge on their exterior surface like all the others, contain within their membranes a second colloid system in which the ambient serum is relatively acid (pH 5.4 instead of 6.9) and contains surface

active proteins showing cationic activity. As colloids, these two systems are quite incompatible and can only co-exist so long as the lutoid membrane keeps them apart. When this membrane leaks or is ruptured there is immediate co-precipitation. This is explicable in terms of charge neutralisation and it has been demonstrated that lutoid serum added to rubber hydrocarbon suspension can reduce the zeta potential of the rubber particles to near zero. However destabilisation occurs before the zeta potential is neutralised completely and this may be due in part to bridging effects. In the particular case of lutoid serum it is thought that the serum contains at least one protein of configuration and charge distribution peculiarly favourable to such a bridging mechanism.

The experiments described in this paper provide a ready explanation for the initial fast phase of latex destabilisation by lutoid serum, and the sudden bursting of a lutoid could certainly induce a local flocculation by the mechanisms proposed. A slow seepage of lutoid serum would encounter a barrier to wholesale destabilisation of rubber, in that the lutoid serum would be co-precipitated with anionic C-serum proteins as fast as it emerged from the lutoids. This is the probable explanation of the protective action of C-serum previously reported.

The observed processes of lutoid decomposition after tapping are explicable on the basis that the lutoid membrane is becoming increasingly permeable. Seepage of C-serum into the lutoid would destroy stability conditions for the colloid system which it contains, so that the interior particles would come out of suspension. Simultaneously proteins would be precipitated and would cause the membrane to thicken. Whatever processes caused the membrane to become less effective as a barrier might be expected to continue to the point of disintegration. Seepage of lutoid serum in the opposite direction would at an early stage reduce the initial negative charge on the membrane exterior, so that in an anionic medium such as C-serum, the lutoids would present a 'sticky' surface, they would tend to adhere together and to other particles. The occurrence of lutoids as aggregates (HOMANS AND VAN GILS, 1948; RUINEN, 1951) and the increase in size of these aggregates as latex ages has often been described (HOMANS AND VAN GILS, 1948; SOUTHORN, 1961).

It is of great interest that reduction of zeta potential of rubber particles by the addition of cationic surfactants or proteins results in fast destabilisation in the form of flocculation, but does not necessarily lead to a true coagulation (irreversible hydrogel) of the rubber. A widely accepted explanation for the coagulation of rubber by acid is that the latex is brought to the isoelectric point of the colloidally protective protein envelope of the rubber particles, *i.e.*, that the zeta potential of the particles is brought to zero. From the present investigation it would seem that this should bring about flocculation but not necessarily coagulation. The inference is that the coagulation following acidification of latex involves additional factors. A ready explanation would be that the adjustment of pH also activates enzymes which fairly rapidly lyse the protective envelope of adjacent rubber particles, permitting true rubber to rubber contacts which fuse into an irreversible gel, or true coagulum.

The authors have regarded latex as a dilute cytoplasm. In any cytoplasm it is evident that a great array of proteins will be required to carry on the cell processes. These proteins may be expected to cover a wide range of isoelectric points so that colloidal interactions are inevitable unless extremes are isolated by physical barriers of some sort. The extensive development of membrane systems in all cells as revealed by electron microscopy is almost certainly relevant in this context.

POST SCRIPT

TATA AND YIP (1968) have now succeeded in isolating protein fractions from B-serum corresponding to individual bands of the starch gel and paper electrophoresis patterns. Band (a) of the starch gel band (dense left hand band of *Figure 1* B-serum) showed strong destabilising activity towards rubber dispersions. B-serum from which the proteins of Band (a) had been separated showed no signi-

ficant activity. It would seem that the proteins of this strongly cationic band are mainly responsible for the effects noted in this paper. Band (a) of the starch gel pattern corresponds to Band (i) obtained by paper electrophoresis of B-serum (MOIR AND TATA, 1960).

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