41. THE PROTEINS OF *HEVEA BRASILIENSIS*

2. ANALYSIS OF A PRODUCT ISOLATED FROM CREPE RUBBER

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It has long been suspected that proteins played some part in the chemistry and technology of latex and raw rubber, but it is only recently that evidence has been obtained as to their exact function. It is now known [Bondy & Freundlich, 1938; Kemp & Straitiff, 1940] that the proteins in latex are the controlling factors in its stability. Thus, the coagulation point of latex corresponds to the isoelectric point of the proteins in solution; dialysed latex is not readily coagulated by acid unless a small concentration of salt is added, a property which is also common to the proteins. When latex is concentrated in the factory by centrifugal methods it is rendered much more unstable, presumably because it has lost a large amount of the protein which acted as a stabilizing agent.

Knowledge of the influence of proteins on the properties of raw rubber has been much more vague. It is known, however [Davis & Blake, 1937], that they do exert a considerable influence on the vulcanizing properties of rubber and that the water absorption of raw rubber depends on the amount of protein present [Skinner & Drakeley, 1931]. More recently Bloomfield & Farmer [1940] have shown that when crepe rubber is fractionated a series of N-free fractions are obtained which are soluble in the usual rubber solvents. The end-fraction which contains the bulk of the N is, however, insoluble. If, however, N-free rubber (prepared from trypsin-digested latex) is used as the starting material then all the fractions are soluble. Gee & Treloar [1940], using the fractions of Bloomfield & Farmer, have shown that the N-free fractions fall into a definite molecular series, whereas the fractions which contain N are radically different in behaviour.

Tristram [1940] described the isolation and analysis of a protein from dried fresh latex films. It was not known what relationship this product bore to the total protein of latex or to the protein which is associated with the hydrocarbon in raw rubber. De Vries [1924] expressed the opinion that latex contained two proteins, only one of which was precipitated with the rubber, but advanced no evidence to prove this view. Bondy & Freundlich [1938] isolated two protein fractions from ammonia-preserved latex and showed that both had isoelectric points within the precipitation range of latex but made no statement as to the presence of a third protein. Kemp & Straitiff [1940] repeating the work of Bondy & Freundlich claimed the presence of a third protein which was not coagulated with the hydrocarbon. Thus, if the findings of Kemp & Straitiff are correct, latex contains three proteins A, B and C, of which A and B are precipitated with the rubber. The preparation extracted from dried fresh latex films [Tristram, 1940] by borate may therefore be a mixture of A, B and C or, more probably, a sample rich in protein C. Unfortunately, none of the above workers characterized his preparation by analytical methods, so that it cannot be said definitely that A, B

and C were separate entities. The present work was accordingly carried out to determine the relationship of this borate-extracted protein to the total protein of latex and to the protein found in raw rubber.

EXPERIMENTAL

The material chosen as the source of protein was the common commercial form, crepe rubber. The method of extraction first tried was that used previously [Tristram, 1940], but no protein was isolated. This suggests either that the protein in crepe rubber is somewhat denatured or that the protein is intimately associated with the rubber hydrocarbon. The latter seems to be more probable in view of the findings of Bloomfield & Farmer [1940] and of Gee & Treloar [1940].

The method of isolation adopted ultimately was based on that of Midgeley *et al.* [1937], since it depended on the dissolution of the rubber from the protein. The method has been modified in accordance with recent work in the above laboratories [Bloomfield & Farmer, 1940].

Method of isolation

500 g. shredded crepe rubber were extracted with acetone, in a drip extractor, for 24 hr. in an atmosphere of N_2 . The residue was dried *in vacuo* and then extracted continuously with light petroleum under N_2 in an apparatus designed by Dr Michael [see Bloomfield & Farmer, 1940]. This procedure removed the 'sol' rubber (ca. 50% total), which was virtually nitrogen-free (0.05–0.02%). The remainder, 'gel' rubber, was coagulated with acetone, dried *in vacuo* and exhaustively extracted with xylene at 60–70°, during which time the mixture was disturbed as little as possible, the organic solvent being replaced by decantation every 24 hr. The residue was next refluxed *in vacuo* with xylene and the insoluble material removed in the centrifuge. The final residue, a gelatinous mass, was extracted successively with xylene, benzene and toluene and then dried *in vacuo*. The dry material was brittle and fibrous; it contained 34–35% rubber, 8.13% ash and 8.9% N; the N content, ash- and rubber-free, was 14.6%. So far it has not been found possible to separate the protein and rubber otherwise than by methods which involve breakdown of the protein, i.e. acid or alkaline hydrolysis. This finding is in keeping with that of Bloomfield & Farmer [1940] discussed above.

Analysis of product

The yield of protein, after correcting for the rubber content, was 2.3 g. or 0.46% of the crepe. Owing to lack of material the analysis (Table 1, 2) could not be made so extensive as those reported previously (Table 1, 1), but it was

Table 1
(Results are given as percentage of total N)

Method:	Dried latex	Crepe rubber
	1 Borate buffer 0.1–0.15	2 Organic extraction 0.46
Yield g. %		
Total N (ash- and rubber-free)	14.95	14.65
Amide-N	7.07	7.18
Tyrosine-N	3.3	3.1
Tryptophan-N	1.2	0.0–0.7
Arginine-N	12.9	13.0
Lysine-N	6.4	6.8
Glutamic acid-N	7.65	Not less than 1.8
Aspartic acid-N	6.5	Not less than 4.1

considered that values for ammonia, tyrosine, tryptophan, lysine and arginine would be sufficient for the purpose of comparison. In addition, the dicarboxylic acids have been investigated qualitatively to test the validity of the claim of Midgeley *et al.* [1937] that glutamic acid is absent from the protein of raw rubber.

DISCUSSION

The results show clearly that the two products have the same contents of ammonia, tyrosine, arginine and lysine, and in view of this agreement it is suggested that either there is only one protein in latex or that there is a mixture of proteins of which preparation 1 is a representative sample. The protein from crepe rubber was different in tryptophan content. One sample contained no tryptophan and a second, prepared from a new sample of crepe, contained 0.7 % of tryptophan-N. It is known that tryptophan is a labile amino-acid, being removed from the protein molecule extremely easily by digestion: it is more probable, however, that, in the present case some change in the specificity of the amino-acid has occurred in the intact protein molecule during the manufacture of the crepe rubber from latex. (NaHSO_3 is often added to latex before coagulation with formic acid and after being coagulated and washed on the rollers the crepe is dried for 2 to 3 weeks in a tropical atmosphere ($30^\circ \pm$).) The loss of tryptophan is being investigated and the preliminary results indicate that NaHSO_3 does affect this amino-acid. Furthermore, proteinaceous material has been isolated from crepe rubber which had been oxidized in the presence of acetic acid [Bloomfield—unpublished experiments] and it has been found that this material contains only 0.2–0.3 % of tryptophan-N whereas the tyrosine-N is 2.8–3.1 %. These results suggest that the change in the specificity of tryptophan does take place during the manufacture of crepe rubber.

If the above suggestion is correct it follows that this protein or mixture of proteins is precipitated, more or less completely, on the coagulation of latex and is thus present in crepe rubber. Such a finding would be in keeping with the evidence of Bondy & Freundlich [1938] referred to above, but is in contrast to the ideas of De Vries [1924] and Kemp & Straitiff [1940]. It has been observed on numerous occasions [cf. Altman, 1939; Whitby & Greenberg (unpublished)] that some protein does, indeed, remain in the serum after coagulation of the latex. These workers, however, used serum prepared under factory conditions, and it is probable that some of the protein escaped precipitation since the pH would not be rigidly controlled. It is also possible that there are present in latex small amounts of proteinaceous material, probably a degradation product of the native proteins, which remains soluble at the coagulation point of the latex.

That the main bulk of the protein is precipitated is seen from the following experiment. A sample of latex preserved with sodium pentachlorophenate was found to contain 0.136 % protein-N. The dry rubber content was 38.0 %, so that the calculated N content of the rubber, assuming that the whole of the protein is precipitated, should be 0.37 %. A sample of the latex was then coagulated and the rubber creped under semi-factory conditions. The N content of the crepe was 0.37 %.

The analytical results given in Table 1 are not in complete agreement with the qualitative experiments of Midgeley *et al.* [1937], who obtained no evidence for the presence of cystine, tyrosine and glutamic acid in the proteins of raw rubber. The absence of tyrosine has also been suggested by Kemp & Straitiff [1940], who reported that the proteins of latex gave a negative Millon's test. It would appear that the analytical methods used by both sets of workers were inadequate for the purpose.

SUMMARY

A protein (2) has been isolated from crepe rubber and analysed for amide-N, tyrosine, tryptophan and the basic amino-acids. The dicarboxylic acids have been shown to be present.

The results indicate that preparation 2 is of the same composition as preparation 1 [Tristram, 1940].

It is now suggested that preparation 1 is a representative fraction of the total protein of latex.

Loss of tryptophan has been observed from the protein found in crepe rubber and a reason for this loss has been suggested.

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