43. THE PROTEINS OF *HEVEA BRASILIENSIS*

I. ANALYSIS OF A PRODUCT ISOLATED FROM DRIED LATEX

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MUCH work has been done on the correlation between the physical properties and the protein content of rubber latex. Weber [1903] and Freundlich & Hauser [1925] suggested that the rubber globules in latex were coated with protein and that this protective layer influenced the coagulation of the latex [see also Bondy & Freundlich, 1938]. Knowledge regarding the nature and composition of the protein(s) of latex, however, is still incomplete, and with the exception of the work of Belgrave [1925] and Bishop [1927] and the early work of Frank [1914] there have been no detailed accounts of the isolation and differentiation of the individual proteins.

Some attention must be given to the work of Belgrave and Bishop because, apart from qualitative tests mentioned in other papers, they are the only workers who have attempted to elucidate the chemical composition of the proteins of latex. Thus Belgrave [1925] obtained a single product by a method involving the use of alcohol as a rubber precipitant, while Bishop [1927] isolated three products, A, B and C, from serum obtained by the freezing of fresh latex. The results, obtained by the indirect method of Van Slyke, are given in Table 1.

Table 1

Values quoted are in percentage of total protein-N

	Belgrave [1925] 1	Bishop [1927]		
		A (acid)	B (NaCl)	C (heat)
Ammonia-N	7.7	5.65	1.62	5.0
Humin-N	0.5	0.45	0.29	0.14
Diamino-N	34.6	22.6	54.0	10.0
Non-basic-N	59.2	56.9	41.8	83.4
Total	102.0	85.6	97.7	98.5

It can be seen that none of Bishop's results agrees with those of Belgrave, nor would it appear that any combination of them would agree.

The results obtained by Belgrave are of particular interest. From 11 l. latex (D.R.C. 38-40 %) he isolated 8.5 g. protein (N, 11.6 %). This is equivalent to 1.5 g. protein (N, 15 %) per kg. of dry weight (total solids *ca.* 40 %), which amount corresponds very closely to that isolated, under the best conditions, by the present author. Moreover, recent work [Miller, 1935; Tristram, 1939] has shown that the Van Slyke method gives values for basic N which may be misleading, and on these grounds the diamino-N of 34.6 % reported by Belgrave might well represent a true basic N of approximately 20 %, a value found by direct analysis in the present work. Table 2 shows the comparison between the two products.

Table 2

	Belgrave	Author
Yield per kg. D.R.C.	1.5 g.	1.0-1.5 g.
Total N %	11.6	14.95
Humin-N (% of total N)	0.5	3.03
Basic-N (% of total N)	34.6	20.0
Ammonia-N (% of total N)	7.7	7.1

Apart from a recent paper by Midgeley *et al.* [1937] little is known of the amino-acid composition of the proteins of latex, and these workers do not mention the amounts of the various amino-acids found in the protein analysed. The product isolated by them from rubber by fractionation with organic solvents contained 12.5 % N, and they obtained evidence of the presence of glycine, aspartic acid, leucine, proline, arginine, histidine and lysine; while cystine, tyrosine and glutamic acid were reported as being definitely absent. Such an analysis cannot be regarded as satisfactory, and it was decided that a more detailed study of the amino-acid composition might furnish valuable data on the products likely to result from degradation of the protein during various technical processes. At the same time it was hoped that comparative analyses might indicate how many proteins exist in latex.

EXPERIMENTAL

The chief difficulty with regard to the preparation of one, or perhaps more than one, protein from latex lay in the fact that the fresh latex itself was not available, and after experiments with various preserved products it was ultimately decided to use air-dried films prepared in Malaya from fresh latex as the starting material. It was realized that drying may have caused some denaturation but whatever the change it should not have affected the amino-acid composition of the protein(s).

The method employed was to extract the film with a borate buffer, pH 9.2. The dried latex was shredded into thin strips and extracted for 12 hr. (including 3 hr. at 50°) with 0.024 *M* sodium diborate [cf. Lugg, 1939] to which were added 0.4 vol. of alcohol and 0.1 vol. of ether. The extract was filtered through paper pulp with a top layer of silica gel (B.D.H. Hyflo Super Cel) and the protein coagulated by acidifying to pH 4.5-5.0 with 10 % acetic acid. The yield was found to be very variable.

In one experiment approximately 24 % of the total N of the dried latex was extracted, and of this amount about 30 % was precipitated as protein-N, corresponding to 1.2-1.9 g. protein per kg. of dried latex. An attempt was made to obtain increased yields of protein by repetition of the borate treatment after re-shredding the extracted strips on smooth rolls. Much nitrogenous material was thus obtained in solution but the amount of protein therein was negligible. Whether any protein was rendered unavailable by this latter treatment is uncertain, but it is known that mastication does, in fact, produce a change in the protein and it may be that all, or part, of that which remained in the films was denatured on the rolls and could not, in consequence, be extracted by the borate buffer, which, according to Dr J. W. H. Lugg (private communication), will not extract denatured protein. Various other media were tried, including solutions of LiCl, NaCl, Na₂SO₄ and (NH₄)₂SO₄, but none of these extracted any protein.

The protein obtained was perfectly white while wet, but on removing water by means of alcohol it darkened somewhat to a buff powder. The isoelectric

point was not determined accurately, but flocculation from borate solution occurred between pH 4.5 and 5.0. The undehydrated product was soluble in dilute NaOH (pH 8.5), and was precipitated from such a solution by nearly complete saturation with NaCl (the precipitate did not redissolve on dilution), and by one-third saturation with $(NH_4)_2SO_4$. (Bondy & Freundlich [1938] isolated from ammoniated latex a protein which was precipitated from solution by one-third saturation with $(NH_4)_2SO_4$.)

Surface films

A sample of protein which had not been extracted with alcohol and ether was dissolved in $N/20$ NaOH and diluted with borax to make an approximately 0.01 % solution of pH 8.5. This solution was spread on phosphate buffer (pH 7.0) and examined by means of a Langmuir-Adam surface pressure balance. On compression the pressure became measurable at a surface concentration of $ca. 0.4 \times 10^{-7}$ g. protein per $cm.^2$, and rose steadily to reach a value of 14 dynes/cm. at a concentration of $ca. 0.8 \times 10^{-7}$ g./ $cm.^2$. The film was liquid throughout. The areas observed are much smaller than those normally found for proteins [Adam, 1938, p. 87], and furthermore all proteins hitherto examined have given gel films on compression. The behaviour found here is typical of mixed films of protein and fat, and further examination showed that considerable amounts of lipin could be extracted from the protein by means of alcohol and ether. When this had been done, the protein could no longer be spread on phosphate buffer (pH 7.0) from borax solution. An $N/20$ NaOH solution was therefore diluted with 20 vol. of 75 % ethyl alcohol to make an approximately 0.01 % solution, from which films could readily be spread. The limiting surface concentration at which the pressure was first measurable was about 1.0×10^{-7} g./ $cm.^2$ and the pressure rose to 10 dynes/cm. at 1.8×10^{-7} g./ $cm.^2$, while gelation was observed at a pressure of 2 dynes/cm. These observations bring the latex protein into line with proteins previously studied.

Since both the lipin and protein had been extracted from the latex by what was essentially an aqueous solvent it was thought that the lipin might contain phosphatide. Analysis showed, however, that it contained no more than a trace of P and that it consisted largely of glyceride. In one particular experiment a crude product gave 2.9 g. lipin and 1.50 g. of protein. The lipin gave the following analysis:

N	0.07 %
P	Trace
Glycerides	91.65 % (fatty acids 87.7 %)
Unsaponifiable matter	7.14 % (81.4 % precipitated as digitonide)
Total recovered	98.79 %

Homogeneity of the various samples of protein

As stated above the yield of protein varied widely, on occasions being as high as 1.9 g. per kg. of dried latex; and in all 11.36 g. (after thorough extraction with alcohol and ether) were obtained from 9 kg. of dried latex. In view of the large variation in yield it was necessary to check the homogeneity of the products obtained from the different samples of dried latex. Lugg [1937; 1938] states that the tyrosine content of a protein may be estimated with a fair degree of accuracy (2 %), and estimations of this amino-acid were accordingly carried

out on the first sample of protein (0.5 g.) and on the bulk sample (11.36 g.) with the following results:

	Total wt. (g.)	Total N (%)	Tyrosine (%)	Tryptophan (%)
Batch 1	0.50	14.9	6.40	1.25
Bulk	11.36	15.0	6.40	1.35

These values suggest that the various samples of protein were of the same composition.

Analysis of the protein

The protein was analysed for the nine more important amino-acids, viz. tyrosine, tryptophan, cystine, methionine, arginine, histidine, lysine, glutamic acid and aspartic acid, and also for amide-N by micro and semi-micro methods which have been repeatedly checked, using standard proteins, by workers in this laboratory.

Amide-N. The protein was hydrolysed for 3 hr. with 2*N* HCl, and the liberated ammonia estimated by (Kjeldahl) distillation into standard acid.

Tyrosine and tryptophan. Triplicate estimations were carried out using the colorimetric method of Lugg [1937; 1938].

Total S. The method used was the alkali-fusion recommended by Bailey [1937] and Lugg [1938].

Cystine and methionine. These were estimated by Baernstein's method as modified by Lugg [1938].

Basic amino-acids. (Single determination.) These were estimated by Block's [1934] method as modified by Tristram [1939].

Dicarboxylic and basic amino-acids. The modification of the original Foreman [1914] method used in this laboratory [cf. Chibnall *et al.* 1940] requires 20–50 g. of protein, but owing to lack of material it was necessary to carry out the estimation on no more than 4.0 g. of protein. Trial experiments carried out on casein showed that, using 2.5 g. of protein, approximately 90% of the glutamic and aspartic acids were recovered as hydrochloride and copper salt respectively, and that arginine and lysine could be accurately estimated in the filtrate from the alcoholic precipitation of the calcium salts (Table 3).

Table 3. *Analysis of casein*

Method	Wt. protein g.	Glutamic acid %	Aspartic acid %
Macro (Foreman)	70	21.0	6.2
Micro modification	2.5	18.7	5.76
		19.1	5.76
	Percentage recovery	90.4	93.0
		Arginine %	Lysine %
Tristram [1939]	2.5	3.76	6.02
The same, applied to dicarboxylic acid filtrate	2.5	3.84	5.93
	Percentage recovery	ca. 100	

The technique was applied to the rubber protein and the values obtained for the dicarboxylic acids (Table 4) were given a correction which assumed that the recovery observed was the same as in the case of casein. The results of these analyses are given in Table 4; for the purpose of comparison, the limiting values for leaf proteins analysed by similar methods (from Chibnall [1939]) have been included.

Table 4. *Analysis of latex protein*

	Protein of <i>Hevea</i> latex		Various leaf proteins Amino-acid as % protein (N=17%)
	N of amino-acid in % total protein-N	Amino-acid as % protein (N=17%†)	
Ammonia	7.07	1.46	0.97- 1.17
Arginine	12.9*	6.90	7.1 - 8.2
	13.0		
Lysine	6.4*	5.68	4.6 - 6.2
	6.4		
Histidine	1.0	0.63	1.0 - 1.6
Glutamic acid	7.65	13.68	11.4 -14.0
Aspartic acid	6.5	10.5	7.9 - 8.8
Tyrosine	3.3	7.24	4.8 - 6.1
Tryptophan	1.2	1.49	2.1 - 2.4
Methionine	0.62	1.12	2.2 - 2.7
Cystine	0.70	1.02	1.4 - 2.2
Total accounted for	47.4	49.72	
% Total N in product analysed	14.95	—	12.8 -15.0
Total S	0.70		

* Estimation carried out on the filtrate from the Ca salts of the dicarboxylic acids.

† The N content of the latex protein has been taken as 17% for the purpose of comparison with the leaf proteins [cf. Chibnall, 1939].

DISCUSSION

The nine amino-acids estimated represent 47.4 % of the total N. Since great difficulty was experienced in the estimation of cystine and methionine the values for these amino-acids must be regarded as minimal only.

It can be seen from Table 4 that the protein has many points of resemblance with those of typical leaf proteins; this is particularly marked in the case of the diamino-acids and the dicarboxylic acids, and the similarity suggests that there may be some relationship between the proteins in the leaves and in the latex of *Hevea brasiliensis*. Samples of protein have now been isolated from the leaves of *H. brasiliensis* and it is hoped to make comparative analysis in the near future.

Midgely *et al.* [1937] stated that the protein present in rubber did not contain cystine, tyrosine and glutamic acid. The protein analysed by the author, however, contained 12% glutamic acid, 6.4% tyrosine and at least 1.0% cystine (calculated as amino-acids on the protein). It is realized, however, that the two proteins in question may not be identical, since it is not claimed that the protein isolated by the extraction of dried latex with borate buffer represents the whole of the protein in latex. Further investigation is accordingly in progress to find what relation the protein so far isolated bears to the whole protein(s) of latex. It has often been stated [de Vries, 1924] that latex contains two proteins and that on coagulation one of these (A) remains in the serum while the other (B) is believed to be adsorbed on the precipitated rubber. If this theory be correct then dried latex will contain both A and B, and borate buffer might be expected to extract A preferentially. Crepe rubber on the other hand should contain B only. Preparations of protein from both these products are now being made by the method of Midgely so as to test this supposition by comparative amino-acid analysis.

SUMMARY

A protein has been isolated from dried *Hevea* latex and analysed for amide-N, tyrosine, tryptophan, cystine, methionine, the basic amino-acids and the dicarboxylic acids.

The dicarboxylic acids were estimated in 4.0 g. protein; experiments on casein having shown that, under such conditions, approximately 90% of the glutamic acid and aspartic acid were recovered as crystalline derivatives. It was also found possible to estimate arginine and lysine in the filtrate from the calcium salts of the dicarboxylic acids.

The protein is, in many respects, similar to those found in leaves of other plants (Table 4).

Further work is in progress to investigate the relationship of the isolated protein to the total protein of latex.

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