

The Modification of Polypeptides in *Hevea brasiliensis* Latex Resulting from Storage and Processing

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Introduction

Latex is exuded from the specialized laticifers situated just beneath the bark but outside the phloem tubes and interspersed with the sieve tubes of the rubber tree trunk on cutting or tapping. The proteins are present in three major systems, the lipid membrane surrounding the rubber particle (mean diameter ca. 400 nM), the cytoplasmic or "C" serum (the liquid phase of latex), and the lutoidic tonoplast, a vacuolar organelle characteristic of *Hevea* latex and some other plant latices that also has the properties of a lysosome. Two other organelles of the latex vessel or laticifer, the ribosomes and the plastid-like Frey-Wyssling particles, are exuded in latex but although of considerable biochemical importance they are of no significance in latex technology (1).

Latex preserved with ammonia (high ammonia latex [HAL] containing 0.7% ammonia or low ammonia latex [LATZ] containing 0.2% ammonia and other anticoagulants) lacks the integral lutoidic tonoplast but instead contains within the serum the released contents of that organelle. The preserved latex used by manufacturers is enriched in rubber content; freshly tapped latex containing 30–35% rubber is treated with ammonia and creamed, usually by centrifugation in a Sharples-type Alfa Laval cream separator, to give a latex concentrate containing about 60% rub-

ber (2). Subsequent to the original observation by Nutter (3) of the capacity of articles manufactured directly from natural rubber latex, usually by dipping, to provoke an immediate or type I (Coombs and Gell) allergic response, it has been shown repeatedly that these articles have a surface layer that contains protein derived from latex, usually readily removed by washing with detergent, physiological saline, or water. Thus three classes of protein preparation have been used to provoke *in vivo* and analyze by *in vitro* methods the allergic response to rubber latex articles, viz. C serum from fresh rubber (usually reconstituted previously lyophilized material), serum from ammoniated latex (usually HAL), and glove extracts. To appreciate the possible protein nature of the allergens it is necessary to understand the protein composition of the nonrubber phase of fresh latex and the polypeptides associated with rubber particles and their relation to the polypeptides of ammoniated latex serum and rubber particles and the aqueous extracts of rubber latex goods.

Results and Discussion

Polypeptides of Fresh Latex C Serum

The first electrophoretic analyses of serum from fresh latex and of serum from ammoniated latex were reported by Roe and Ewart (4) who, using the Tiselius electrophoresis apparatus, demonstrated the presence of seven resolvable components in sera obtained by thawing frozen latex (this therefore would have contained proteins liberated from the luteoid particles); treatment of latex with 1% ammonia for several months resulted in a serum in which only three major components could be discerned. Archer and Sekhar (5) analyzed a latex serum preparation prepared by a similar procedure, by paper electrophoresis and also succeeded in resolving seven components, six of which appeared to be analogous to those of Roe and Ewart; exposure of the latter to 1.2% ammonia for 15 d reduced the number of resolvable components to about 4. The analysis of fresh C serum from latex (which does not contain the protein liberated from the luteoid particles) was not markedly different from that of the obtained by freeze-thawing.

The use of more modern methods of gel electrophoresis, such as that in starch or polyacrylamide, does not produce analyses that give a very clear resolution of native latex serum proteins.

The proteins of C serum are, however, highly immunogenic in both rabbits and sheep. Polyclonal polyspecific antisera raised in both of the species have been used to resolve the proteins of C serum by immunoelectrophoresis. The results of two-dimensional immunoelectrophoresis of the C serum proteins of the clone most frequently grown in Southeast Asia, viz. RRIM 600, using polyclonal antisera raised in four different rabbits are shown in Fig. 1A. Immunoelectrophoretic analysis of four other clones using the same antiserum are shown in Fig. 1B. There are clearly some differences in the relative amounts of the various components in C sera from the different clones; there may be a seasonal variation in the protein composition of C serum but this has not been studied. Comparison of the polypeptide composition of C sera from different clones by SDS polyacrylamide gel electrophoresis (Fig. 2) shows that there are some small differences between the clones, but again the possibility of seasonal variations has not been investigated. It has been suggested that the relatively recent reporting of Type 1 allergy to latex goods may arise from the change to more modern *Hevea* clones as a source of latex. Latex from Thailand and Malaysia is probably mostly derived from the clone RRIM 600 and that from Sri Lanka from clone PB 86. These clones are related, RRIM 600 being a product of the cross PB 86 $\times$ TJ 1, and there is little difference in the polypeptide composition of C sera from these three clones.

A characteristic feature of the two-dimensional immunoelectrophoretic pattern of Fig. 1 is the major protein peak, third in order of cationic mobility. Excision of the precipitin line owing to this component and its use for subsequent immunization gave a monospecific polyclonal antiserum 99. Figure 3A shows the result of a two-dimensional immunoelectrophoresis of latex C serum using the antiserum 99 that reacted maximally with fractions of C serum proteins eluting from a calibrated Sephacryl S 300 column in a volume corresponding to mol wt of about 80 kDa as adjudged by the Laurell rocket technique (6). Immunoblots of C serum using this antiserum showed a reaction with a 40 kDa polypeptide (Fig. 4). The same result was obtained whether or not β -mercaptoethanol had been included in the sample buffer (not shown) indicating that the 80-kDa native protein was a dimer, the 40 kDa subunits being linked by noncovalent forces.

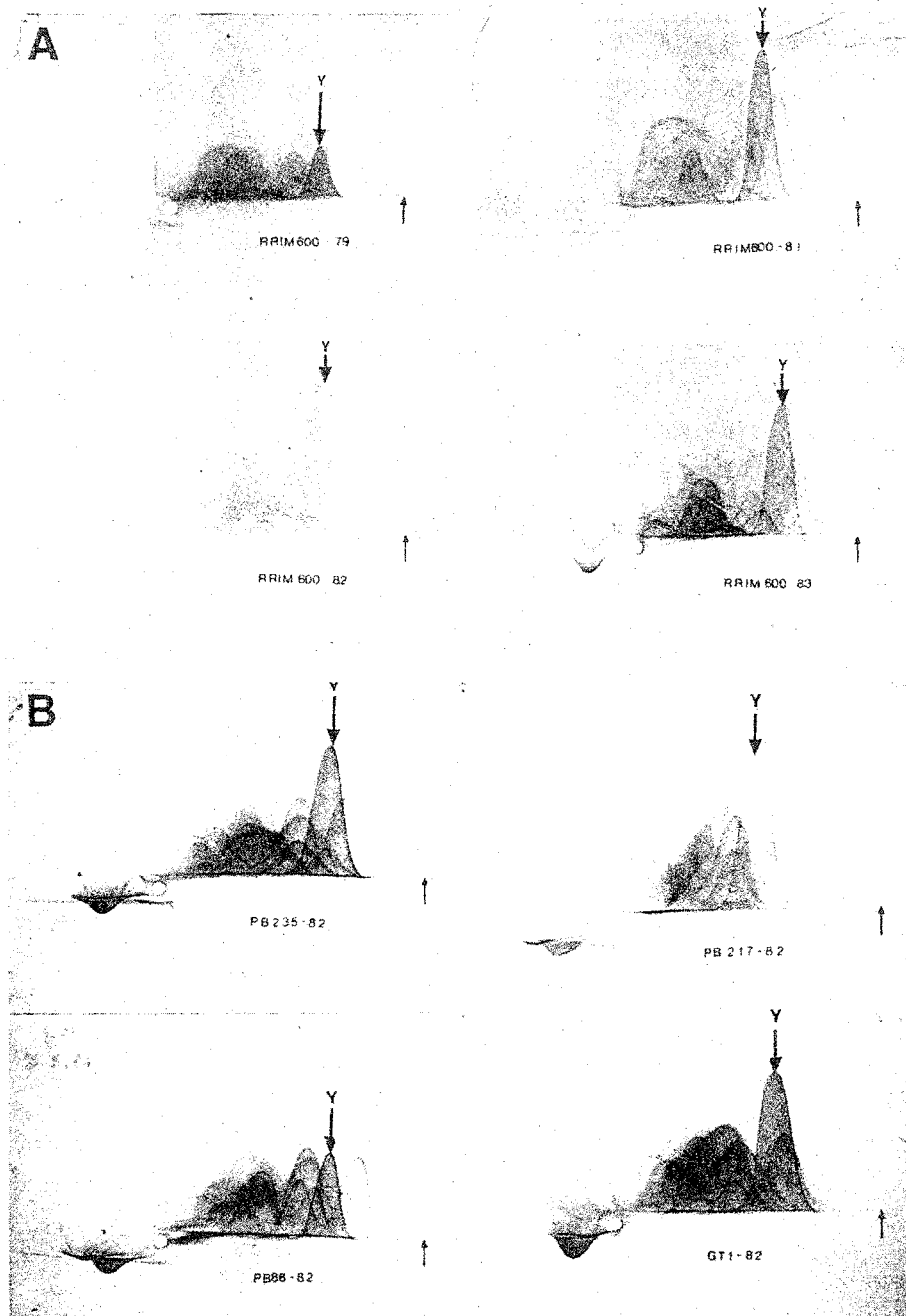


Fig. 1. (A) Two-dimensional immunoelectrophoresis of lyophilized C serum proteins of clone RRIM 600 detected by four different rabbit polyclonal antisera. (B) Two-dimensional immunoelectrophoresis of lyophilized C serum proteins from four different commercial *Hevea* clones using the same rabbit polyclonal antiserum.

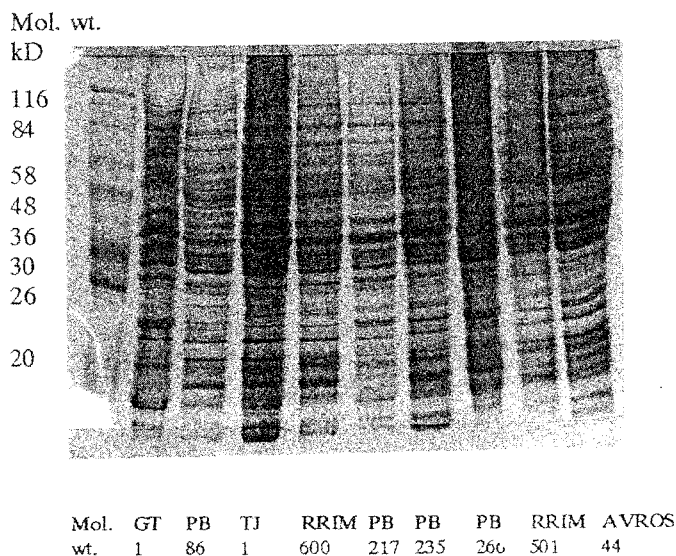


Fig. 2. SDS polyacrylamide gel electrophoresis (10% acrylamide) of polypeptides from lyophilized C serum of various commercial clones.

The Polypeptides of Ammoniated Latex Serum

As noted above both Roe and Ewart (3) and Archer and Sekhar (4) showed that, as would be expected, there was extensive modification of the latex serum proteins consequent on treatment with ammonia. On the other hand, Lynn and Clevette-Radford (7) have reported the preparation of an active serine protease, hevain, mol wt 69 kDa, from ammoniated latex showing that at least some biochemical integrity of latex proteins survives preservation and storage.

Figure 5 shows two-dimensional immunoelectrophoresis of HAL serum using a polyclonal polyspecific antiserum (79) to C serum proteins; again it is seen that the proteins have been extensively modified and only about five components can be clearly discerned. The proteins of a major cationic peak persisted. This has been found to give a reaction of identity with peak Y (Fig. 1) of lyophilized fresh latex C serum (result not shown). Two-dimensional immunoelectrophoresis of the HAL serum proteins (Fig. 3B), using the monospecific antiserum 99, shows the mobility of the reacting protein to be slightly greater than that of the corresponding protein in C serum. Comparison of the immunoblots of the C serum and ammoniated latex serum shows the latter to

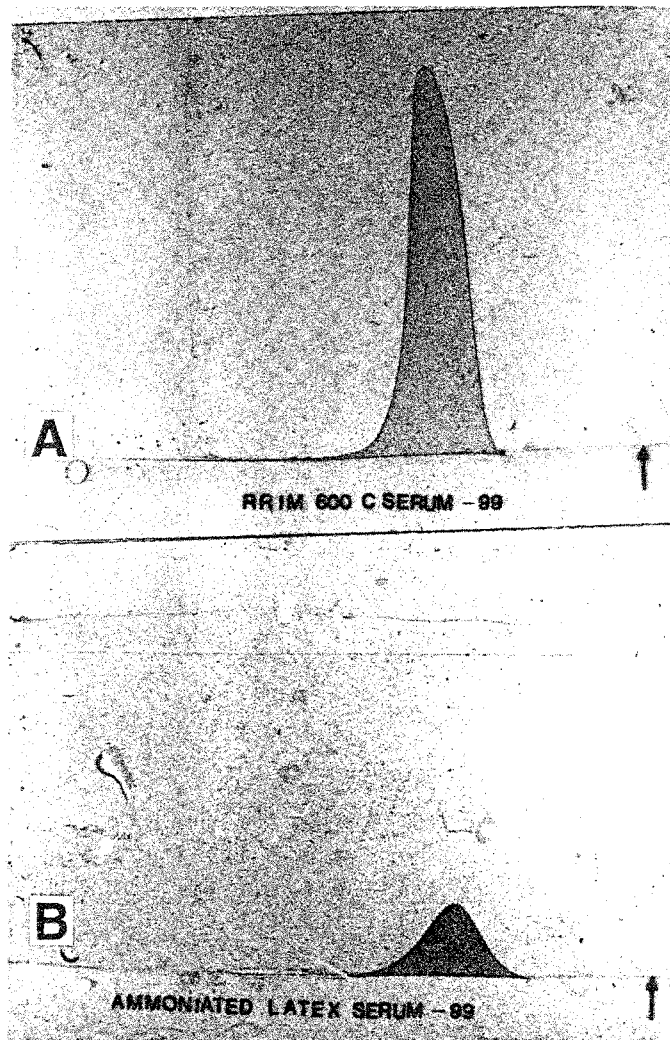


Fig. 3. Two dimensional immunoelectrophoresis of (A) lyophilized C serum proteins of clone RRIM 600 and (B) HAL serum using a rabbit polyclonal antiserum 99 to peak Y.

contain a product of partial proteolysis of the protein with a mol wt of about 26 kDa. There are also some other minor components, one of which has a mol wt somewhat greater than the 40 kDa subunit of the native protein (Fig. 4).

Although SDS polyacrylamide gel electrophoresis of HAL total serum gives a rather indistinct resolution of the polypep-

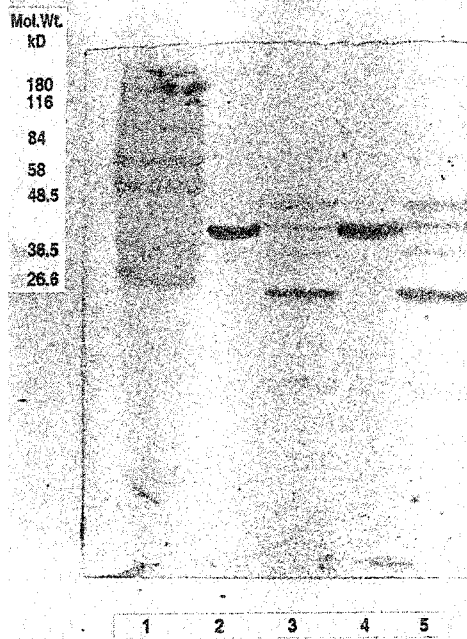


Fig. 4. Immunoblot of lyophilized C serum (tracks 2 and 4), and HAL serum (tracks 3 and 5) reacting with antiserum 99 raised to peak Y of Fig. 1. Track 1 shows mol wt markers.

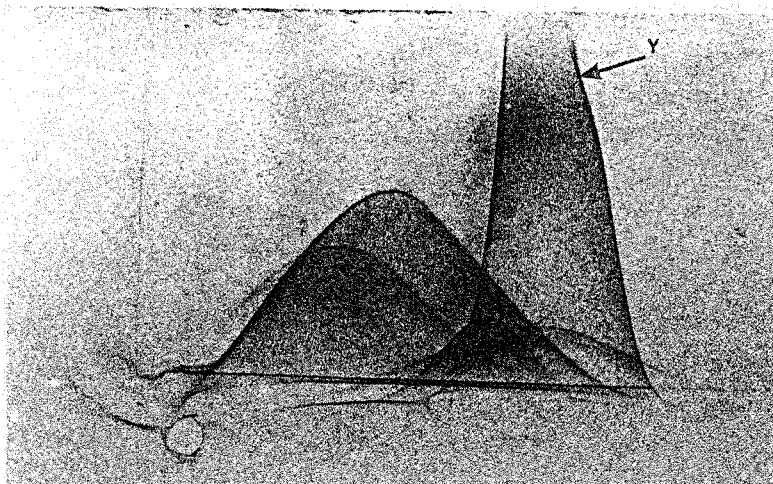


Fig. 5. Two dimensional immunoelectrophoresis of HAL serum using a rabbit polyclonal antiserum (79) to lyophilized C serum proteins of clone RRIM 600.

tides, fractionation of this material on a calibrated Sephacryl S 300 column separates, in the higher mol wt fractions, some material that appears to cause the indistinct background, so that electrophoresis of the fractions eluting in the larger volumes shows the resolution of several polypeptides (Fig. 6). It is, however, apparent that the polypeptides are interacting with the matrix—a complex mixture of polypeptides being eluted in volumes somewhat greater than the void volume. The presence of a polypeptide of mol wt about 14 kDa in fractions eluting in volumes corresponding to mol wt of 40–60 kDa is of interest in view of the attribution of allergic properties to a 14-kDa polypeptide (8).

Comparison of ELISA assays of the 80-kDa protein of peak Y on fractions of C serum and ammoniated latex C serum eluted from a calibrated S 300 column using antiserum 99 confirms the reduction in the mol wt of the reacting component attributable to partial proteolysis shown in the immunoblots, but material eluting in the ammoniated latex serum both in the same volume as the native protein and in smaller volumes, corresponding to a higher mol wt, also gives a positive ELISA test, suggesting that the polypeptide may also undergo further polymerization in ammoniated latex (Fig. 7).

The Polypeptides Associated with Rubber Particles

Dennis and Light (9) have obtained serum-free rubber particles by centrifuging the liquid portion of thawed frozen latex and applying the creamed rubber particles to a Sephacryl S 300 column. The rubber particles eluted in the void volume yielded two polypeptides on extraction with 1% SDS, of mol wt 24 and 14.6 kDa, respectively. The 24-kDa polypeptide was selectively removed by washing the particles with 0.01% Triton X. It was reported that the 24-kDa polypeptide was present in the C serum, but the only evidence for this was a band corresponding to that mol wt in the SDS-PAGE analysis of C serum.

When washed rubber particles are applied to a Sephacryl S 1000 column, they can be size-fractionated, the larger particles being eluted first and the smaller particles subsequently, the size being measured in the fixed state by electron microscopy or in suspension by photon correlation spectroscopy. Analysis of the associated polypeptides by SDS polyacrylamide gel electrophoresis shows that the large particles contain very little associated

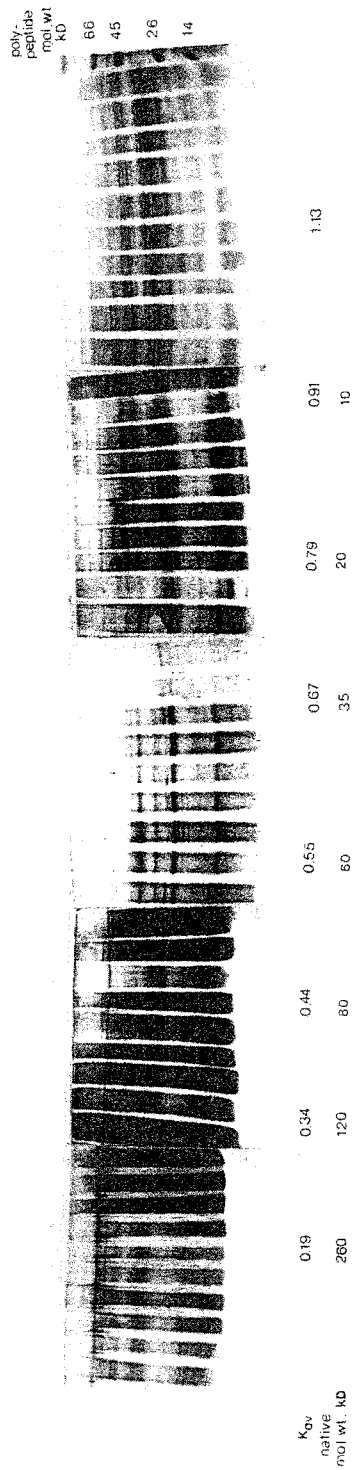


Fig. 6. SDS polyacrylamide gel electrophoresis (12% acrylamide) of fractions of HAL serum eluted from a calibrated Sephacryl S 300 column (93 x 2.6 cm) by 0.1M phosphate buffer pH 7.4.

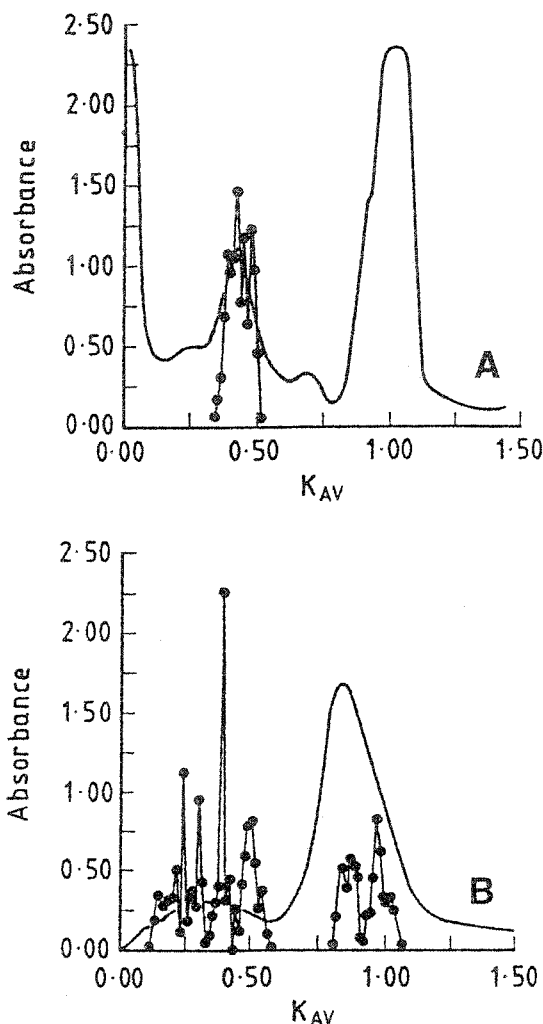


Fig. 7. Detection of the epitopes of the protein of peak Y (Fig. 1) in fractions of fresh latex C serum (A), and HAL serum (B) eluted from the Sephacryl S 300 column as in Fig. 6 by antiserum 99 using the ELISA technique.

polypeptide. The 14.6-kDa polypeptide predominates on the particles, but the smaller particles have both the 24- and 14.6-kDa polypeptides and the very small particles have a large number of associated polypeptides (results not shown). Figure 8 shows the SDS polyacrylamide gel electrophoretic analysis of the polypeptides of the larger rubber particles of fresh latex separated from C serum by elution from a Sephacryl S 1000 column together

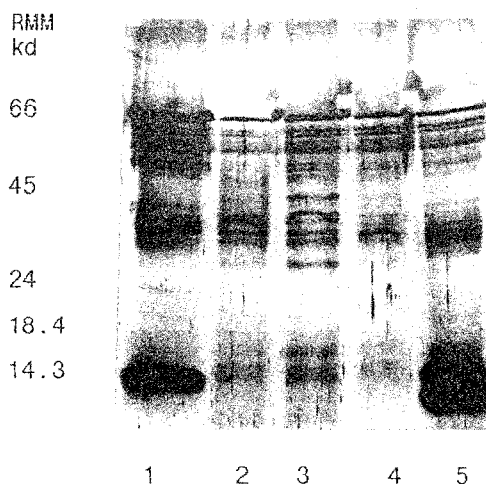


Fig. 8. SDS polyacrylamide gel electrophoresis (12% acrylamide) of rubber particle polypeptides from fresh and HAL preparations and polypeptides extracted from films prepared from prevulcanized latex. Track 1, rubber particles from fresh latex. Tracks 2, film extracts 1–3. Track 5, rubber particles from HAL concentrate. Rubber particles from fresh latex prepared by washing $3 \times$ (0.25M tris buffer, pH 8.0) followed by gel filtration on Sephacryl S 1000. Rubber particles from ammoniated latex concentrate prepared by washing $6 \times$ (0.25M tris buffer, pH 8.0).

with the analysis of the polypeptides of washed rubber particles of HAL. It is of interest that the major 14.6-kDa polypeptide of the particles from fresh latex has been to an extent partially degraded to give a peptide of mol wt about 12 kDa.

The Polypeptides of Glove and Film Extracts

Estimation of the proteins eluted from rubber gloves by extraction with 8 mL phosphate buffered saline/g glove (ca. 1 cm squares) by the Nessler (10), Ninhydrin (11), and Bradford (12) methods gave the results shown in Table 1. The Nessler nitrogen and ninhydrin methods gave results for five brands of surgical gloves that, although different, bore an approximately constant proportion to each other. The associated protein estimated by the Nessler procedure for these five brands ranged from 14–42 $\mu\text{g/g}$ glove, with a mean of 29 μg . The other three brands of surgical gloves gave much higher assays, two having values ten times the other five. The Bradford method gave very much lower results

Table 1
Protein Concentrations in Glove Extracts

Brand	$\mu\text{g protein/g rubber}$		
	Bradford	Nessler	Ninhydrin
A	113	284	1538
B	18.0	85	244
C	11.0	34	98
D	8.6	24	54
E	5.2	42	127
F	4.0	14	42
G	412	139	717
H	1.1	7	12
I	16.7	300	1609
J	4.2	29	82

Extracts were prepared by shaking 1 cm squares of gloves in phosphate buffered saline (8.0 mL/g glove) for 3 h at room temperature. The extracts were concentrated by pressure dialysis before assaying for protein. Brands A, C, D, E, and F were surgical gloves. B, I, and J were surgical gloves marked hypoallergenic. H was a household glove.

and there was not a consistent ratio between the values obtained by this procedure and those obtained by the other two methods. The household gloves gave much lower values of associated protein estimated by the three methods, as would be expected for thicker gloves.

SDS-PAGE electrophoresis of the glove extracts gave analyses showing varying degrees of resolution of the polypeptides (Fig. 9A). Some brands gave similar patterns showing a number of polypeptides between 55–66 kDa, others in the range 24–40 kDa and some lower mol wt polypeptides between 14–24 kDa. In regard to the implication of a 14-kDa polypeptide in the allergic reactions to latex, it is noteworthy that all the extracts studied contained polypeptides of around this mol wt. Gloves of a few brands had elutable polypeptides in excess of 66 kDa mol wt.

The comparison of the polypeptide composition of rubber particles from fresh latex and HAL with that of rubber films formed from vulcanized latex (Fig. 8) shows that the film extracts contain a polypeptide of about 14 kDa, similar to that associated with the rubber particles.

Immunoblots of four of the glove extracts against antiserum 99 showed a clear reaction with two of the extracts, a smear with a third, and no reaction with a fourth (Fig. 9B). In another immu-

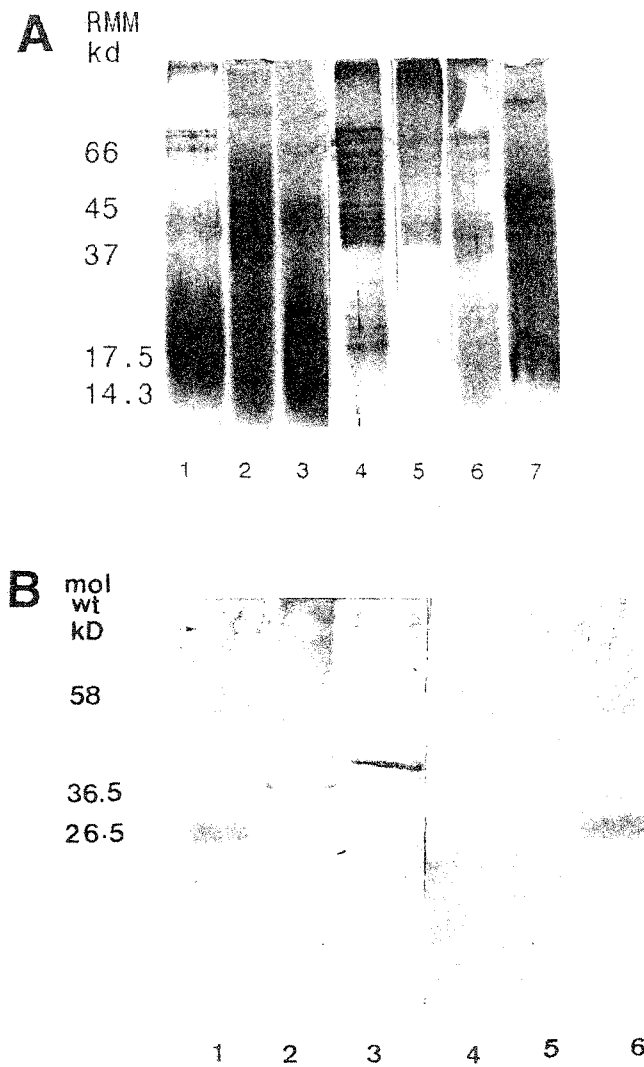


Fig. 9. (A) SDS polyacrylamide gel electrophoresis of extracts of examination and surgical gloves obtained by washing with phosphate buffered saline. Tracks 1–7, brands A–G. (B) Immunoblots of glove extracts vs. antiserum 99 containing antibodies to peak Y, Fig. 1.

noblot (results not shown), a further two of the extracts were found to react with the antiserum. The polyspecific polyclonal antiserum (79) as used for the analyses shown in Fig. 1 to the native C serum proteins gave continuous smears on the immunoblots of

glove extracts. It was remarkable that, when the extract of one brand of gloves (B) was subjected to amino acid analysis, no cysteine or methionine could be found in the hydrolysate.

Conclusions

It is clear that the proteins of fresh latex undergo considerable changes in their antigenic properties as a result of preservation with ammonia and subsequent manufacture. Antigens reacting with an antiserum to a major 80-kDa protein of fresh latex were, however, found in both ammoniated latex serum and in some glove extracts, indicating the persistence of some epitopes of a native protein through preservation and the manufacturing processes. The polypeptides of glove extracts varied both quantitatively and qualitatively with different brands and it appears that their nature and concentration are to an extent a function of the manufacturing processes. There were no consistent differences between gloves marketed as hypoallergenic and those not thus designated as far as surface proteins likely to provoke type I allergic responses are concerned.

Acknowledgments

I am grateful to the Malaysian Rubber Producers Research Association for defraying some of the research expenses connected with this work. I am also indebted to K. Syo of this department for the protein analyses and to Catharine Peyramaure of the Institut National Agronomique Paris Grignon for carrying out the ELISA assays.

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