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Latex Allergy Studies: Location of Soluble Proteins in Latex Examination Gloves

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The cause of soluble proteins eluting mainly from the inner surface of latex gloves, as compared with the outer surface, was investigated. This differential protein elution from the glove surfaces did not arise from the latex coagulant or cornstarch used in glove manufacture. Eleven commercial brands of latex examination gloves showed greater uptake of the protein stain, Naphthalene Black, on the inner glove surface than on the outer surface, reflecting the trend in the amounts of proteins extractable from the two surfaces. Light microscope examination of the cross section of the gloves showed a distinct region of soluble proteins stained at the inner surface. The staining was much reduced in samples where the proteins had been earlier removed by leaching with water. Abrading 2 μm (3% of the glove thickness) from the inner glove surface resulted in a 54% decrease of extractable soluble proteins. These results suggested that differential leaching of proteins from the two glove surfaces was not due to the existence of a barrier to protein diffusion. It was best explained by the migration of proteins to the inner glove surface during manufacture.

There have been an increasing number of reports and papers drawing attention to the problem of Type I allergic reactions to dipped rubber products caused by latex proteins[1-5] Protein is naturally present in latex of *Hevea brasiliensis* which is the commercial source of natural rubber. *Hevea* latex contains about 0.95% protein of which about 27% is in the rubber fraction, 48% in the serum fraction and 25% in the bottom fraction[6]. Some of the latex proteins are soluble while others are associated with organelles such as the rubber particles. The primary cause of allergic reactions[5,7,8] is traced to water soluble proteins. These could include insoluble latex proteins that are solubilised during the course of manufacture of the latex products.

A disparity in the amount of proteins leached from the two glove surfaces has been reported, with about 95% of the proteins coming from the inner surface[9]. This trait might be explained by one of two possibilities:

- Soluble proteins are concentrated at the inner surface of the glove during manufacture
- Proteins are dispersed throughout the thickness of the glove, but they are prevented from being eluted from the outer surface by some form of impermeable barrier.

An understanding of how differential elution of glove proteins is brought about could facilitate the formulation of more effective strategies to overcome the latex protein problem of the latex industry. In the present study, the cause of the differential elution of proteins was investigated. In the course of the investigation, the location of soluble proteins in latex gloves was established by *in situ* staining.

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MATERIALS AND METHODS

Preparation and Electrophoretic Separation of Latex Proteins

Latex from clone RRIM 600 was collected in chilled containers and centrifuged at 19 000 r p m. (43 000 g) in a Sorvall RC5B centrifuge for 1h to obtain three main fractions: a zone of rubber cream that was centripetally separated, a heavy 'bottom fraction' comprising mainly the lutoids, and the C-serum in between. The rubber fraction was resuspended in 30% sucrose and re-centrifuged. Proteins of the membranes of the rubber particles were solubilised and extracted by adding an equal volume of detergent comprising 0.1% Triton-X 100 and 1% sodium dodecyl sulphate (SDS) to the rubber cream. The mixture was vortexed and then centrifuged. The rubber particle membrane proteins were recovered in the aqueous fraction. To prepare B-serum, the lutoids of the 'bottom fraction' were ruptured by alternate freeze-thawing about six times. B serum was then recovered as the supernatant after re-centrifugation[10]. The B serum, C-serum and rubber particle membrane proteins were dialysed in tubing with a molecular weight cut-off of 1.5 kDa.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of B-serum proteins, C-serum proteins and rubber particle membrane proteins was carried out according to Laemmli[11]. The gels were stained with Naphthalene Black or Coomassie Blue. The stains (0.1%) were prepared in a mixture of 50% methanol and 10% acetic acid. Destaining was carried out in a mixture of 10% methanol and 7% acetic acid.

Preparation of Latex Films

Latex films were prepared from pre-vulcanised (PV) latex either by dipping or by casting. Where coagulants (10% calcium nitrate and 7% calcium carbonate in water) were used, it was difficult to prepare smooth, even films by casting and dipping was preferred. Where coagulants were absent, casting was preferred since straight-dipped films were too thin for use. Bioabsorbable cornstarch was applied by dipping the films in an 8% slurry. The latex films were dried at 100°C for 15 min.

Assessment of Uptake of Stains by Insoluble Latex Proteins

To investigate the stain uptake by insoluble glove proteins, soluble proteins were first largely removed from samples of fresh latex, high ammoniated (HA) latex and PV latex by diluting the latices with water (1:4) and centrifuging to recover the rubber cream. The cream(estimated to contain only about 2% serum) was smeared onto a glass slide, air dried and stained for 3 h with each of the protein stains, Naphthalene Black or Coomassie Blue, and then destained.

Surface Staining of Latex Gloves

To evaluate stain uptake on the inner and outer glove surfaces, fingers were cut from the test glove samples and some were turned inside-out as appropriate. A glass marble was inserted into the finger and the cut end of the finger was then tied. Fingers were immersed in each of the protein stains, for 3 h before destaining. This method was adopted for the surface examination of stain uptake as it enabled the glove samples to be stained on either side separately. Evaluation of stain intensity could not be carried out on samples stained on both sides because of the translucency of the gloves. The stained samples were then air dried.

Samples (stained and unstained) were photographed using Ektachrome colour slide film. The photographic slides were cut into rectangular pieces to fit the cuvette of the Beckman DU-65 spectro photometer. A spectral scan of the slide showed that absorbance was maximal at 655 nm. Absorbance readings of slides of the stained gloves were therefore taken at 655 nm and corrected by subtracting the absorbance of the corresponding unstained gloves.

Staining and Microscopy of the Glove Cross-section

Pieces of glove samples from the palm and finger areas that were intended for the examination of stain uptake on their cut edges (cross sections) were immersed in stain so that all surfaces were stained at the same time. Other details were as given above. The cross-sections of stained samples were observed under the bright field of an Olympus SZH Zoom stereo microscope.

Surface Abrasion of Latex Glove

Abrasion of the glove surface with sand paper was carried out using an electrical orbital sander. Micrometer screw gauge measurements (mean of 10 readings) showed that only about 2 μm of the surface was removed as further abrasion tended to tear the glove.

Protein Quantitation

Protein quantitation by a modified Lowry procedure was carried out as previously described[12].

Materials

Latex examination gloves were obtained from retail outlets and manufacturers. Gloves are turned inside-out in the factory when they are stripped from the former. The inner and outer surfaces in this report refer to the finished product; hence, the surface originally in contact with the glove former is designated the outer surface.

HA latex contained 0.75% ammonia and 60% dry rubber content and MR grade PV latex were obtained from a commercial source. (HA and PV are the source materials used in glove manufacture.) Protein stains and dialysis tubing were purchased from Sigma Chemical Company.

RESULTS AND DISCUSSION

Influence of Cornstarch and Latex Coagulant on Differential Protein Elution

To understand the reported differential elution of proteins from the inner and outer glove surfaces[9,13], attention was directed to the different conditions to which the two glove surfaces were subjected during glove manufacture. Prior to dipping into latex, the glove former is first coated with a coagulant preparation which is commonly a mixture of calcium nitrate and calcium carbonate. Hence, the glove surface next to the former (i.e. the outer surface of the finished product, since gloves are turned inside-out during stripping) comes into direct contact with the coagulant. Cornstarch is normally applied dry or in slurry form on the product before it is stripped from the dipping former; it ends up on the inner surface of the finished glove. Investigations were made to check whether: (a) cornstarch adsorbed and retained high levels of proteins onto the inner glove surface. This protein might then be readily more released and passed on to the glove user than if the starch were not used; (b) the coagulant applied to the dipping former rendered the outer glove surface impermeable to the outward diffusion of protein resulting in the very low levels of proteins eluted from this surface.

Protein was eluted from cornstarch powder that was brushed from a brand of commercial latex gloves by extracting 1 g powder in 30 ml distilled water. Similar extractions were made from unused cornstarch powder obtained from the manufacturer of the gloves. Negligible amounts of soluble proteins were found in the eluate of unused cornstarch whereas 0.21 mg protein/g powder was detected in cornstarch recovered from gloves. While this observation showed that the cornstarch powder adsorbed protein[14,15] as noted previously, the protein level was not sufficiently high to account for the amounts of protein eluted from the inner glove surface.

Calcium nitrate has been shown to precipitate latex proteins[16] and the latex coagulants applied to dipping formers might hence inhibit the free outward diffusion of soluble proteins from latex films. Dipped and cast films were prepared with the full complement of latex, coagulant preparations (applied to the filming support) and cornstarch (applied later to the free surface of the film), and with these components removed

selectively from the formulations. The results showed that the differential elution of latex proteins was maintained in all cases (*Table 1*); hence, the coagulant preparation and cornstarch were not primarily responsible and the coagulant did not form a barrier impermeable to protein.

Differential Staining of Soluble and Insoluble Protein

Latex proteins are either tightly bound to the membranes of rubber particles or organelles in the latex[17,18], or are soluble proteins found in the latex sera (B-serum and C-serum). Some of the soluble proteins (which may include membrane proteins solubilised by ammonia[17]) remain in the finished product after the manufacturing processes that include leaching in water. It has been estimated that insoluble proteins make up at least 94% of the total glove protein[13]. Hence, in any attempt to stain and examine the soluble proteins of latex glove *in situ*, it is important that they are differentially stained from the insoluble proteins. Otherwise, it would not be possible to distinguish the small amounts of soluble proteins (less than 6%) from the predominance of insoluble proteins which are mainly the rubber particle membrane proteins and which, presumably are distributed throughout the thickness of the glove.

To assess the stain uptake by soluble and insoluble *Hevea* latex protein, SDS-PAGE was carried out on solubilised rubber particle membrane proteins, B-serum proteins and C-serum proteins prepared from fresh latex. As to be expected, all these proteins were readily stained with Naphthalene Black and Coomassie Blue. Hence, no differential staining was observed between the soluble serum proteins and the extracted solubilised rubber particle membrane proteins prepared from fresh latex.

Rubber cream was recovered from undiluted and from 4X-diluted fresh, HA and PV latices by centrifugation. When smears of these rubber creams were stained with Naphthalene Black and Coomassie Blue, only the cream from undiluted fresh latex was appreciably stained, with the colour intensity increasing on standing after destaining. Much of this staining could have arisen from soluble serum proteins remaining in the rubber cream rather than from the rubber particle membrane proteins. Markedly reduced stain

TABLE 1. EFFECT OF COAGULANTS AND CORNSTARCH ON THE DIFFERENTIAL ELUTION OF PROTEIN FROM LATEX FILMS

Components of latex film	Method of filming	Film thickness (mm)	Protein (μg sq cm) eluted from surface away from the former (A)	Protein (μg sq cm) eluted from surface adjacent to the former (B)	Ratio A:B
Latex compound + calcium nitrate + calcium carbonate + cornstarch	Dipped	0.20	19.58	0.36	54
Latex compound + calcium nitrate + calcium carbonate	Dipped	0.18	13.76	0.27	51
Latex compound + cornstarch	Cast	0.34	50.20	0.45	112
Latex compound	Cast	0.31	91.17	0.56	163

Protein results are the means of two readings.

uptake was observed when the cream was prepared from diluted fresh latex where the residual serum was minimal. Therefore, unlike the *extracted solubilised* rubber membrane proteins that were readily stained, the same proteins of fresh, HA and PV latices were poorly stained *in situ* (*Figure 1*). It was thus possible to differentiate the lightly stained insoluble rubber particle membrane proteins in latex gloves from the intensely stained soluble proteins that were extractable from the gloves (*Figure 2*).

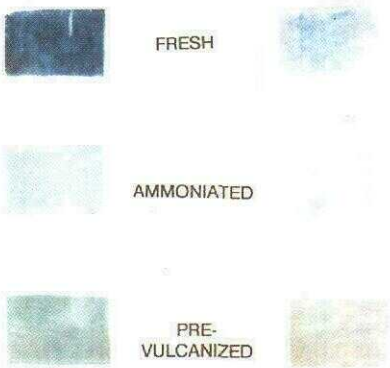

Figure 1. Smears of latex cream stained with Naphthalene Black. Rubber cream that were obtained from latices that were not diluted (left) contained about 15% serum. Rubber cream samples from latices that were diluted (right) contained about 2% serum.

Surface Staining of Gloves

While latex gloves could be stained by both Naphthalene Black and Coomassie Blue, better results were obtained with the former. For some reason, several brands of glove samples could not be satisfactorily stained

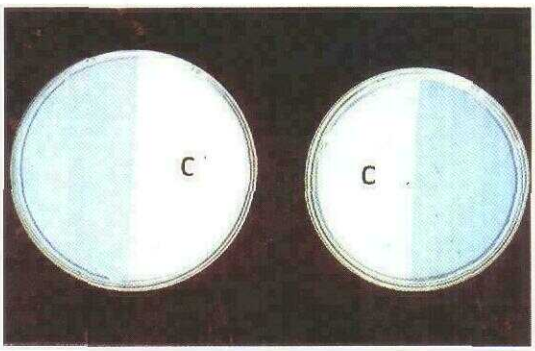

Figure 2. Eluted glove proteins incorporated into agarose (58 μg/ml) stained with Naphthalene Black (left) and Coomassie Blue (right). C = Control portion containing no protein.

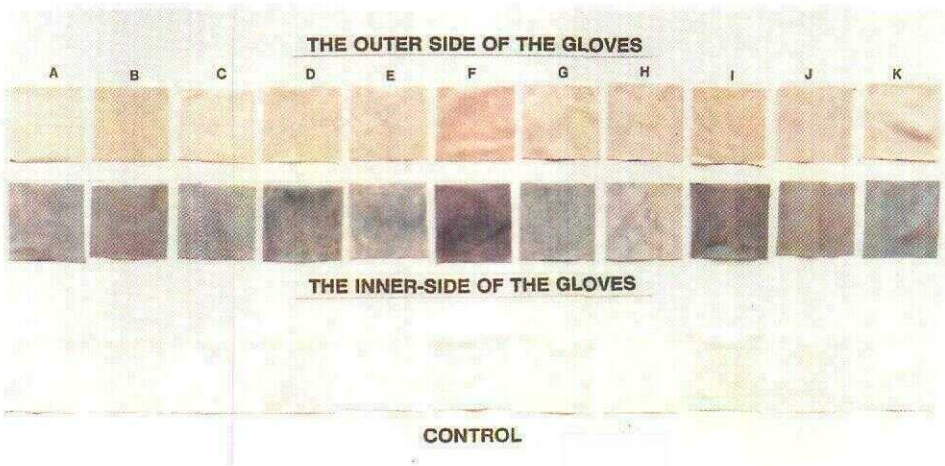

Figure 3. 'Finger' staining of eleven commercial brands of gloves with Naphthalene Black. The outer surfaces (top row) and inner surfaces (middle row) were stained separately. Control samples (bottom row) were unstained.

with Coomassie Blue Hence, Naphthalene Black was mainly used in further experiments

In the eleven brands of gloves studied, greater Naphthalene Black stain uptake was observed on the inner glove surface as compared with the outer surface (*Figure 3*) This disparity in stain uptake reflected the trend in the amounts of soluble proteins leached from the two glove surfaces[9] Nevertheless, the discrepancies between stain intensities on the two surfaces, as quantified by the absorbances at 655 nm, were not as large as when the extractable proteins were assayed On the average, the ratio of proteins eluted from the outer and inner glove surfaces was 1 25 while the ratio of the photometric absorbances was only 1 3 (*Table 2*) This may be partly explained by the fact that insoluble proteins on both surfaces were also stained, although to a much lesser extent Moreover, other non-proteinaceous constituents of the latex formulation might also pick up the stain The photometric readings did not correlate closely with the extractable soluble proteins in the different gloves (results not presented) Hence, stain intensity could not be used as a reliable quantitative comparison of soluble proteins between different brands of gloves

Microscopy of Stain Uptake in Gloves

Examination of the stained cut edge of the glove sample showed that staining was confined to the edge of the cross-section, at the inner surface, the remaining of the cross-section showed little protein staining (*Figure 4*) This indicated that soluble proteins were concentrated mainly at the inner surface of the glove The same pattern of staining was observed in all the eleven brands of gloves studied To confirm that the stained region was indicative of soluble protein and not an artifact, some gloves samples were first leached in water to remove soluble proteins before staining In such instances, greatly reduced staining was observed (*Figure 5*) The results obtained from staining the glove sections were in agreement with the inference by Dalrymple and Audley [13] that soluble glove proteins were located at or near the surface of the film

Effect of Abrading the Glove Surface

Further confirmation that soluble proteins were located on the inner glove surface was obtained by abrading the glove surface lightly with sandpaper using an orbital sander The surfaces of the treated sample were then eluted with water and the eluants assayed for protein

TABLE 2 COMPARISON OF DISTRIBUTION OF EXTRACTABLE PROTEIN ON INNER AND OUTER SURFACES OF GLOVES DETERMINED BY STAINING AND LOWRY ASSAY

Glove	Stain intensity (absorbance at 655 nm)		Ratio of stain intensity	Ratio of proteins eluted
	Inner surface	Outer surface	(Inner outer surface)	(Inner outer surface)
A	1 31	0 33	4 0	22 3
B	1 06	0 28	3 8	41 0
C	0 91	0 26	3 5	19 9
D	0 85	0 28	3 0	16 5
E	0 81	0 13	6 2	7 6
F	0 76	0 41	1 9	32 3
G	0 74	0 16	4 6	5 2
H	0 70	0 35	2 0	35 7
I	0 69	0 40	1 7	53 8
J	0 54	0 31	1 8	29 3
K	0 43	0 36	1 2	15 2
Mean	0 80	0 30	3 1	25 3

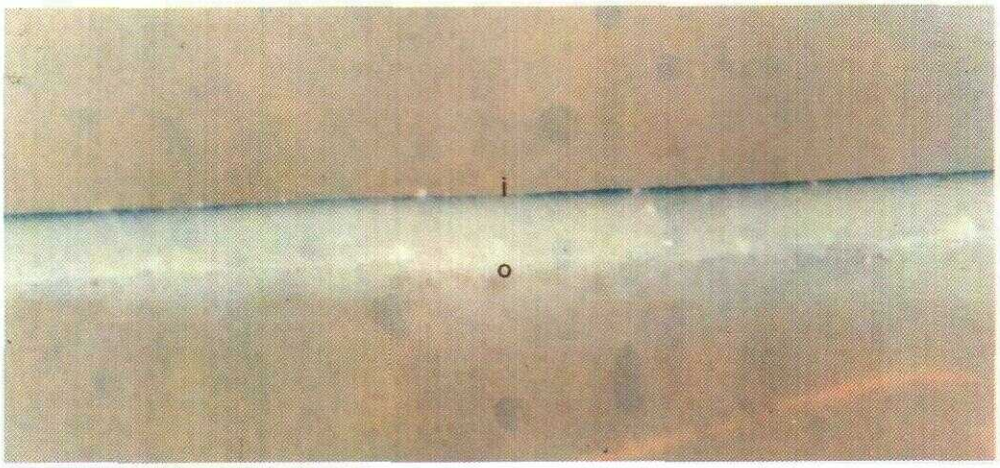

Figure 4. Cut edge of glove (Brand ('C') seen under the microscope. i = inner surface, o = outer surface. Naphthalene Black staining (× 80).

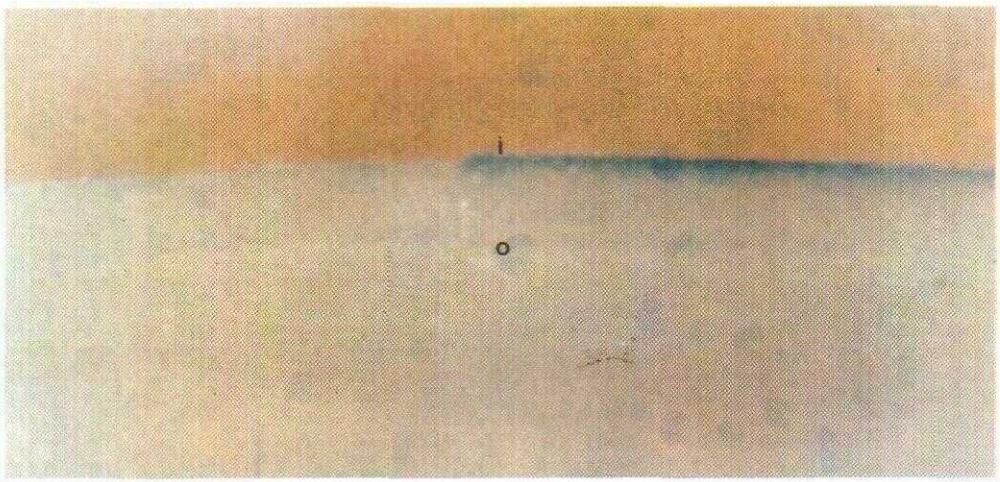

Figure 5. Cut edge of leached (left) and unleached (right) glove (Brand 'D') seen under the microscope. i = inner surface, o = outer surface. Naphthalene Black staining (× 80).

Removal of only 2 μm off the surface (representing about 3% of the glove thickness) resulted in a 54% decrease of extracted soluble proteins from that surface. On the other hand, abrading the outer glove surface did not change extractable proteins greatly (*Figure 6*). These observations were consistent with the proposition that the soluble proteins were concentrated at the inner surface of the latex glove.

CONCLUSION

The results from the various experiments carried out suggested that differential leaching of proteins from the two glove surfaces was not due to the existence of a barrier impeding protein diffusion from the outer surface. It was best explained by the migration of proteins to the inner glove surface during manufacture.

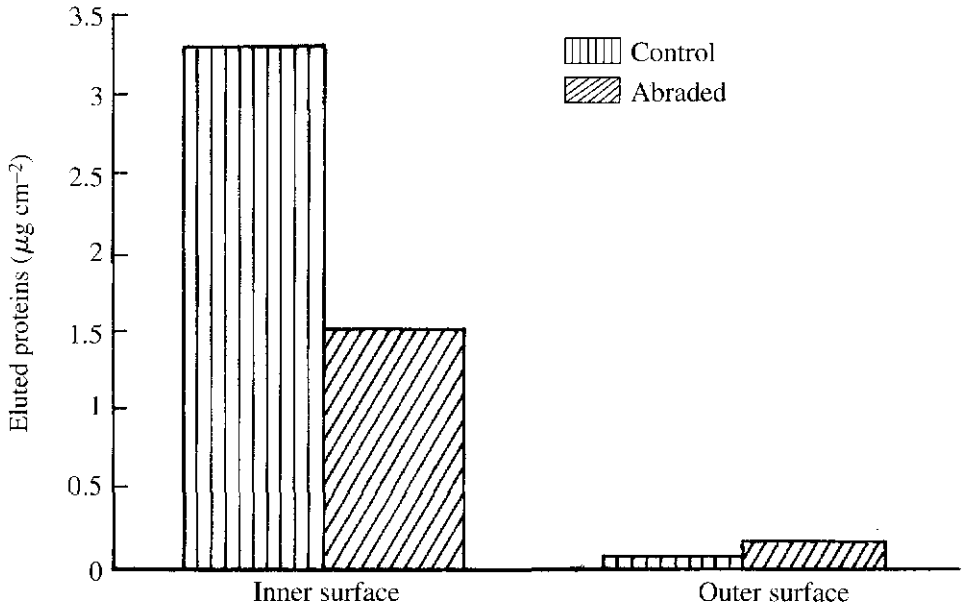

Figure 6. Effect of abrading approximately 2 μm from the inner and outer glove surfaces on the amount of proteins extractable from the surfaces.

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