J. nat. Rubb. Res., **8(2)**, 154 – 161

SHORT COMMUNICATION

Latex Allergy Studies: Differential Leaching of Soluble Proteins from the Inner and Outer Surfaces of Natural Rubber Latex Examination Gloves

H.Y. YEANG* AND FARIDAH YUSOF*

In a study of twelve commercial brands of natural rubber latex examination gloves, a marked disparity was observed between proteins leached from the inner and outer glove surfaces. On the average, about twenty-seven times more protein was leached from the inner surface than from the outer surface. Relatively high levels of proteins were leached from the inner surfaces of the glvoes tested (mean = 7.83 $\mu g\ cm^{-2}$) and there was considerable variability between different brands (standard deviation = 4.27 $\mu g\ cm^{-2}$). On the other hand, proteins leached from the outer glove surfaces were relatively very low (mean = 0.33 $\mu g\ cm^{-2}$) and consistent between brands (standard deviation = 0.17 $\mu g\ cm^{-2}$). By turning the latex glove inside-out before use, the user would benefit from a reduction in exposure to allergenic latex glove proteins by an order of twenty to twenty-five fold (95%–96%). It is suggested that health workers wear two pairs of gloves, with the inner pair turned inside-out. This interim approach to avoid allergenic glove proteins is simple and practical.

Type I (IgE-mediated immediate) allergic reactions arising from contact from natural rubber latex products have been attributed to latex proteins leached from their surfaces[1]. In the most severe cases, an anaphylactic shock that is potentially life-threatening results. In recent years, considerable attention has been drawn towards characterising the nature of the allergic reaction and the protein allergens responsible. At the same time, much effort has been expended in devising ways to decrease the soluble proteins in natural rubber latex products, especially examination gloves which are extensively used throughout the world. Soluble proteins in the source material may be diminished by the use of multiple-centrifuged or enzyme (protease)-treated latex[2,3,4]. However, the simplest method of protein reduction is to leach the gloves on the production line[2,3]. Further protein removal is possible where dry leaching is also practised[2,3] or where the leaching is carried out in a protease solution[4].

Another very effective approach for reducing soluble proteins is by chlorination[2,3]. It is believed that chlorine renders the proteins insoluble or it forms an impermeable barrier that prevents proteins migrating to the glove surface[2]. Steam treatment of gloves has been reported to reduce diffusible soluble proteins[5] while coating of the latex product with an impermeable film has also been proposed to control the release of proteins[3]. A successful approach towards reducing the exposure of allergenic proteins to the glove consumer needs be one that is practicable. It should be technically feasible and should not affect the technical properties of the finished product. The process should not add substantially to the cost of production and preferably should not require extensive alterations to the existing infrastructure and process of glove manufacture. It is no simple matter to meet all these conditions as each approach often has its own drawback. For example, steam treatment might

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

decrease the stability and strength of the glove[3] while enzyme treatments and surface coat applications would certainly add to the cost of production.

This report, presents a study on the leaching characteristics of proteins from the inner and outer surfaces of natural rubber latex gloves. It is hoped that the results of this study would help devise strategies towards alleviating the exposure of the glove user to allergenic proteins. In the meanwhile, they could provide an interim measure for the avoidance of latex glove proteins.

MATERIALS AND METHODS

Fourteen brands of natural rubber latex examination gloves were initially acquired without pre-selection for this study. Two brands were subsequently left out of the study because the beadings on the gloves rolled towards the outer surface rather than the inner surface; thus, the gloves could have been turned inside-out by the manufacturer and could have received additional treatment. Of the remaining twelve brands, nine were bought from retail outlets while the remaining three were commercial stocks from manufacturers. Test samples of gloves were sampled from dispenser boxes. From their appearance and texture, it was assumed that all the gloves were unchlorinated.

The inner and outer surfaces of the gloves refer to their orientation in the finished product. As gloves are turned inside-out when they are stripped from formers during manufacture, the inner surface is the surface of the latex film away from the glove former while the outer surface is the surface adjacent to the former during manufacture. Leaching of

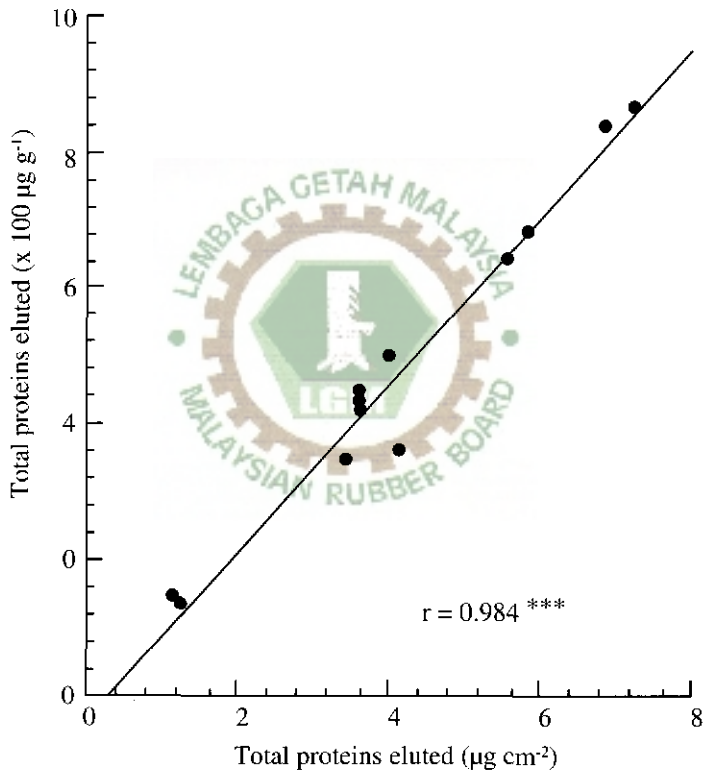

Figure 1. Correlation between total proteins eluted expressed per unit weight of glove and per unit surface area of glove.

proteins from test pieces of latex gloves (both surfaces) was carried out as described previously[6] Pieces measuring 7 × 7 cm square were cut from the palm portion of each glove and two sample pieces were leached in 30 ml of water in a 10 cm diameter container at room temperature for 3 h The glove pieces were turned over after 90 min and the leachate was swirled in the container every 30 min To leach proteins from either the inner or the outer surface of latex gloves, 15 ml of water was pipetted into an uncovered 9 cm diameter petri dish and a piece of glove cut from the palm portion was affixed over it and sealed with tape such that either the inner or outer surface faced the inside of the petri dish The petri dish was then inverted so that the glove piece was at the bottom and the water lay on its surface for 3 h The petri dish was swirled once every 30 min Proteins were leached from either the inner or outer surfaces of the fingers of latex gloves by cutting out the fingers and filling them with water (after turning them inside-out where appropriate) The fingers were tied off with thread at the cut ends and placed on an orbital shaker for 3 h In all comparisons of proteins leached from the inner, outer and both surfaces of gloves, separate glove samples from the same box were used The elution treatments were duplicated

Purification, concentration and quantification of leachate protein were as previously described[6] The leachates were centrifuged to remove insoluble matter and then purified and concentrated by precipitation with trichloro acetic acid (TCA) and phosphotungstic acid (PTA) Protein assay was based on the Lowry reaction[7] and all procedures from the acid precipitation step onwards were duplicated

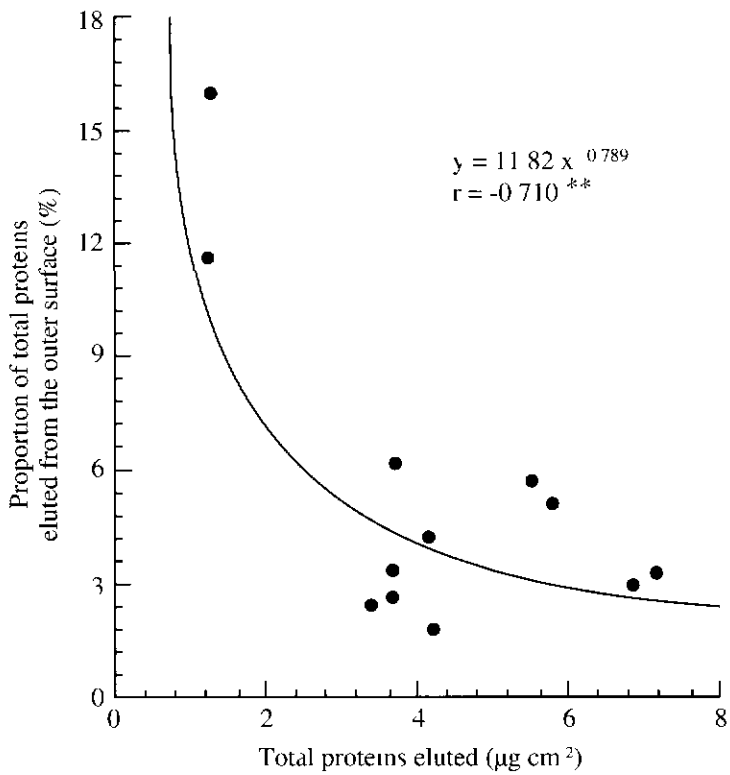

Figure 2 Correlation between total proteins eluted and the proportion of proteins eluted from the outer surface of the glove

RESULTS

The proteins eluted from both surfaces of twelve brands of glove ranged from 129 μg proteins per gramme glove to 876 $\mu g\ g^{-1}$. The mean value was 491 $\mu g\ g^{-1}$ and the median was 449 $\mu g\ g^{-1}$. The different brands of gloves were of dissimilar thicknesses (135–240 μm) and this gave rise to varying weights per unit surface area. When eluted protein was expressed as microgramme protein per square centimetre of the eluted surface, mean and median values of 4.15 $\mu g\ cm^{-2}$ and 3.77 $\mu g\ cm^{-2}$ respectively were found. The range was from 1.18 $\mu g\ cm^{-2}$ to 7.15 $\mu g\ cm^{-2}$. The differences in glove thickness between brands did not greatly affect the relative levels of eluted proteins expressed either as protein per unit weight or per unit area; a strong correlation ($r = 0.984$***) was found between the two *(Figure 1)*.

Departing from the usual practice of immersing glove pieces in the eluting medium (and thereby eluting proteins from both surfaces of the sample), proteins were eluted separately from the inner and outer surfaces. The amounts of protein leached separately from the two surfaces of the gloves are presented in *Table 1*. A mean of 7.83 $\mu g\ cm^{-2}$ proteins was leached from the inner glove surface. In comparison, very much less proteins were eluted from the outer surface, giving a mean of only 0.33 $\mu g\ cm^{-2}$. The ratio of proteins eluted from the inner and outer surfaces determined individually for the twelve brands averaged 1: 26.7 *(Table 1)*.

When protein eluted from the outer surface was expressed as a percentage of the total protein eluted from both surfaces, the outer surface proteins accounted for a mean of only 5.36% of the total protein. Even this value was somewhat inflated because the percentage values increased markedly in gloves that already had very low total proteins as in

TABLE 1. SOLUBLE PROTEINS LEACHED FROM INNER AND OUTER SURFACES OF LATEX GLOVES (PALM AREA)

Glove	Proteins leached from inner surface ($\mu g\ cm^{-2}$)	Proteins leached from outer surface ($\mu g\ cm^{-2}$)	Ratio of proteins from inner and outer surfaces	Outer surface proteins as % of total (inner + outer surface) proteins
A	15.14	0.76	19.9	4.78
B	12.93	0.40	32.3	3.00
C	12.49	0.35	35.7	2.73
D	9.49	0.23	41.3	2.37
E	9.14	0.17	53.8	1.83
F	8.57	0.52	16.5	5.72
G	6.48	0.29	22.3	4.28
H	6.15	0.21	29.3	3.30
I	5.61	0.37	15.2	6.19
J	4.51	0.11	41.0	2.38
K	1.97	0.26	7.6	11.66
L	1.52	0.29	5.2	16.02
Mean	7.83	0.33	26.7	5.36
Median	7.53	0.29	25.8	3.79
Std. deviation	4.27	0.17	14.8	4.29

Brands K and *L (Table 1)*. A more representative figure could be found in the median (3.79%) rather than the mean. The greater the amount of total proteins eluted from the glove, the smaller was the proportion of protein recovered from the outer surface *(Figure 2)*. Hence, in general, the inner surface contributed overwhelmingly to the protein that was recovered when both surfaces of the glove were leached. (Ironically, the inner surface is the surface of the latex film on the dipping former that comes into contact with water during the washing process in glove manufacture.) Not surprisingly, there was a strong correlation (r = 0.904***) between total protein (eluted from both surfaces of the glove) and protein eluted only from the inner surface *(Figure 3)*. There was also a relationship between total protein eluted and protein from the outer surface, but the correlation was poor (r = 0.500, P < 0.1).

The proteins eluted from the two surfaces of a brand of glove were correlated (r = 0.600*, *Figure 4*), but irrespective of the amount of protein leached from the inner surface, the amount eluted from the outer surface was consistently low for all twelve glove samples. The standard deviation for the samples was only 0.17 μg cm^{-2} compared with 4.27 μg cm^{-2} for the inner surface.

The proportions of proteins leached from the inner and outer surfaces of latex examination gloves were examined in the finger portions of four brands. Discrepant amounts of proteins were eluted as observed with the palm portions of the gloves *(Table 2)*.

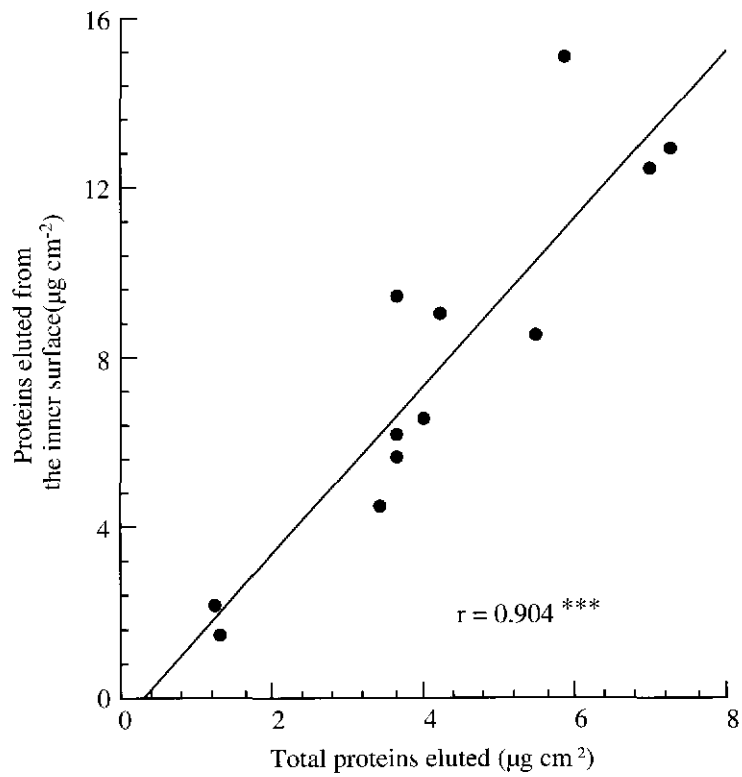

Figure 3. Correlation between total proteins eluted and proteins eluted from the inner glove surface.

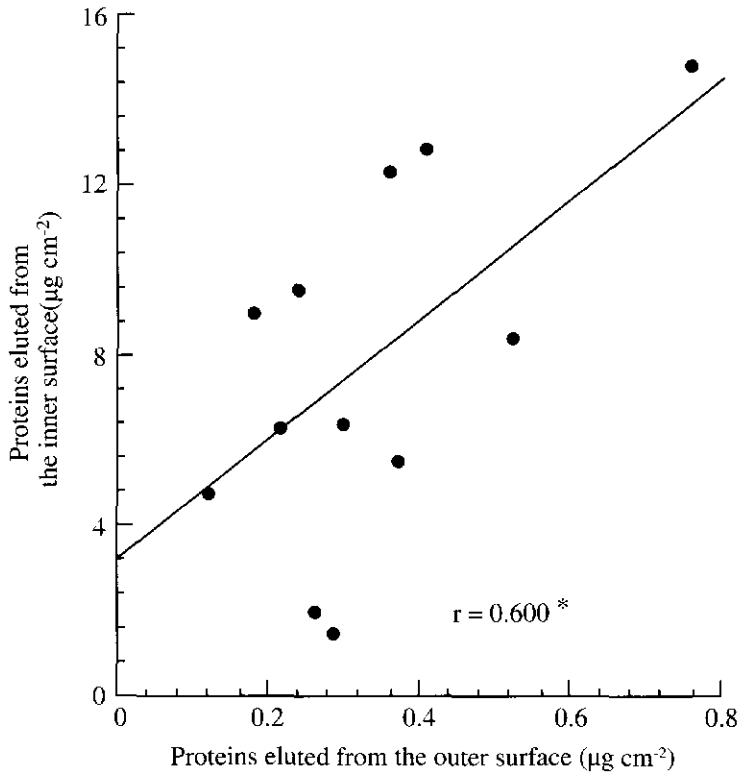

Figure 4. Correlation between proteins eluted from the outer and inner surfaces.

DISCUSSION

Proteins leached from both surfaces of the twelve brands of gloves had a median of 449 $\mu g\ g^{-1}$. This was higher than the median of 150 $\mu g\ g^{-1}$ for latex gloves that was reported previously[6]. The earlier value had been based on gloves from various commercial sources and had included chlorinated gloves and some non-production gloves made from low-protein latices.

The leaching of protein from the inner and outer surfaces of the glove had been studied by Dalrymple and Audley[8] using a different experimental approach. Extracting from the entire glove, they found that more proteins were leached from the outer surface, but the opposite result was obtained from the finger portions of the glove. In the present study, the consistent discrepant leaching of proteins from the two surfaces was demonstrated in the palm portions of the twelve brands of gloves and confirmed in the finger portions of four of the brands. As all twelve brands of gloves tested in this study showed a marked disparity in protein leached from the inner and outer glove surfaces, it is probable that this characteristic is common to latex gloves in general.

The very marked discrepancy in proteins leached from the inner and outer glove surfaces can be exploited to alleviate the exposure of the user to allergenic glove proteins. By simply **turning the glove inside-out**, the user reduces exposure to protein by 96% in the general case. In the present study, this figure was substantially lower only in the brands of gloves that were already very low in overall leachable proteins. It is significant that not only were the levels of proteins eluted from the outer glove surface very low, but the variation between brands was

TABLE 2 PROPORTION OF PROTEINS LEACHED FROM THE INNER AND OUTER SURFACES OF THE FINGERS OF LATEX GLOVES

Glove	Ratio of proteins from inner and outer surfaces	Outer surface proteins as % of total (inner and outer surface) proteins
A	28.6	3.38
B	18.3	5.17
C	22.8	4.21
E	46.5	2.10

extremely low despite the differences in latex sources and production methodologies. Hence, there is a very good chance that a user using *any* brand of glove, even one with which he has no previous experience, will be exposed to a relatively low level of latex proteins if the glove is turned inside-out. Such a practice should go a considerable way towards ameliorating the allergy problem in latex gloves. This solution involves no cost to either consumer or producer and it does not require the glove manufacturing process to be changed in any way. What is perhaps required is an information label informing the consumer that there is more protein on the inner glove surface and that persons allergic to glove proteins are advised to turn the glove inside-out when using them. The precaution of turning the glove inside-out alone may be sufficient for consumers (e.g. those who handle harzardous materials) whose gloved hands do not come into contact with other persons. Those who do (e.g. medical personnel) and who are not allergic to glove proteins should *not* turn the gloves inside-out so that the persons in contact with the gloves are not exposed to the high protein. If such users are themselves allergic to glove proteins, it is proposed that they wear two layers of gloves, the first layer turned inside-out and the second layer not so, in order that the high protein surfaces are sandwiched in between. Despite using two pairs of gloves, glove consumption will not be increased because only the outer glove needs to be changed between patients.

The control of protein diffusion from latex products by coating with an impermeable film has been proposed[3]. Should this strategy be considered for latex gloves, it might only be necessary to coat the inner glove surface where most of the protein accumulates. As this is the latex film surface that is positioned away from the dipping former, it would not be necessary to strip the glove off the former for the coating process; an on-line second dip might suffice.

Further work is needed to understand the distribution of soluble proteins in latex gloves and to elucidate the mechanism responsible for their leaching behaviour. In the meantime, the inversion of latex gloves before use is simple and practical interim method that can be adopted for the *avoidance* of exposure to allergenic proteins from latex examination gloves.

ACKNOWLEDGEMENT

The assistance of Cik Norliza Ishak in the protein assays is gratefully acknowledged. Puan Hafsah Mohd. Ghazaly supplied some of the glove samples.

Date of receipt: February 1993
Date of acceptance: May 1993

REFERENCES

1 TOMAZIC, V.J., WITHROW, T.J., FISHER, B.J. AND DILLARD, S.F. (1992) Latex Associated Allergies and Anaphylactic Reactions. *Clin. Immunol. Immunopath.*, **64(2)**, 89.

2 SUBRAMANIAM, A. (1992) Reduction of Extractable Protein Content in Latex Products. *Proc Int Latex Conf Sensitivity to Latex in Medical Devices, Baltimore, Maryland*, 63.

3. TRUSCOTT, W (1992) Manufacturing Methods Sought to Eliminate or Reduce Sensitivity to Natural Rubber Products. *Proc. Int. Latex Conf Sensitivity to Latex in Medical Devices, Baltimore, Maryland,* 65

4 NIELS, E (1992) Enzyme Application in Latex Device Production to Eliminate Allergic Reactions to Latex Medical Devices *Proc. Int Latex Conf: Sensitivity to Latex in Medical Devices, Baltimore, Maryland,* 34

5 LEYNADIER, F, TRAN XUAN, T AND DRY, J (1991) Allergenecity Suppression in Natural Latex Surgical Gloves *Allergy,* **46(8),** 619

6 FARIDAH YUSOF AND YEANG, H.Y (1992) Quantification of Proteins from Natural Rubber Latex Gloves *J. nat. Rubb. Res* , **7(3),** 206.

7. LOWRY, O.H , ROSEBROUGH, N.J , FARR, A.L AND RANDALL R.J (1951) Protein Measurement with the Folin Phenol Reagent. *J Biol Chem* , **193,** 265

8. DALRYMPLE, S.J AND B.G AUDLEY (1992) Allergenic Proteins in Dipped Products. Factors Influencing Extractable Protein Levels. *Rubber Developments,* **45(2/3),** 51.