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Allergic Responses and Levels of Extractable Proteins in NR Latex Gloves and Dry Rubber Products

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With the awareness of Type I allergy, improvements of latex-dipped products, particularly the medical gloves, invariably involve the reduction or removal of their residual extractable protein fraction containing the allergens. Although extensive studies are in progress to identify these allergens, it is still not clear how the allergic response so elicited in hypersensitive persons by these allergens is related to the quantity of the extractable protein level present in the products. This paper examines this relationship with reference to latex gloves.

Extractable protein content of a total of 39 different glove samples, determined by the Rubber Research Institute of Malaysia modified Lowry microassay procedure, is shown to range from < 0.020 mg/g to > 1 mg/g. Their allergic responses in latex sensitive persons (a total of 59) are evaluated by means of the skin-prick test. Results demonstrate that higher extractable protein contents are always associated with positive allergic responses, while very low extractable protein levels tend to exhibit weak or no allergic reaction.

Similar studies have also been carried out with 16 dry natural rubbers of various commercial grades and five rubber products including cut threads manufactured via processes quite different from those of latex-dipped articles. Findings reveal that they not only have extremely low extractable protein contents (< 0.020 mg/g – 0.034 mg/g), but also show negligible or no allergic responses when skin-prick tested on a total of 28 latex hypersensitive persons. It may therefore be concluded that dry natural rubber products are free from the protein allergy problem reported for some latex products.

Unlike Type IV allergy, the Type I hypersensitivity experienced by some latex sensitised persons is not induced by compounding ingredients added during processing, such as thiurams, mercaptobenzthiozoles (MBT) and carbamates. This allergic reaction is caused by some residual water extractable proteins present in latex products[1,2,3]. Recent onset of this hypersensitivity reported in the West is, to a certain extent, believed to be due to the sudden upsurge in demand of latex gloves (due to 'AIDS' scare) and the subsequent increased production of gloves which unfortunately contained high levels of the allergenic proteins. The latter apparently arose from inadequate leaching during manufacturing. It is therefore likely that this has caused sensitisation to some users, particularly people who are atopic. One logical approach towards overcoming the allergy problem would thus be to ensure future production of latex products contain minimum quantity of the residual extractable proteins.

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Presently, threshold levels of these proteins below which relatively few sensitised people are affected are not known. The extent of protein reduction in latex products is hence of great interest, particularly to the manufacturers. Work is therefore undertaken to study the relationship between the levels of extractable proteins in latex examination gloves and their allergic responses in latex sensitised patients.

The protein allergy problem has so far been confined to latex-dipped products[4]. However, it is learned that recently there is a 'smear' campaign against NR cut-threads, capitalising on the latex allergy issue. In view of this, the extractable protein contents and allergenicity of some dry rubbers and dry rubber products are also investigated.

PROTEIN ALLERGY IN NR LATEX GLOVES

Extractable Protein (EP) Content

It has been well demonstrated that the EP content of latex products varies, according to the processing conditions the products are subjected to during manufacturing. For example, it increases after compounding and when vulcanised or dried at elevated temperature[5,6] of 100°–120°C. It decreases, on the other hand, when leached in water, either during the wet-gel stage or the dry-film stage after vulcanisation/drying[5,6], as well as after chlorination of the products[7]. Hence, the EP values can range from as high as more than 1mg/g rubber for the unleached and untreated sample to as low as 0.03mg/g or less for the well leached or chlorinated samples, as shown by both the modified Lowry microassay[8] and the SE-HPLC method[9].

Currently, extensive studies are being carried out in different countries attempting to identify the various allergenic proteins in latex. Unfortunately, much of such information is still lacking, except that these allergenic proteins could be detected in their different molecular weight fractions or bands, depending on experimental techniques used[4]. Furthermore, it is not clear if the allergic response is related to the quantity of total EP, although some direct relationship has been implied in the case of latex films[10]. This relationship is now being re-examined by studying the EP levels in latex gloves, and evaluating their allergic responses in sensitised patients using the 'skin-prick' test.

Skin-prick Test

One simple and rapid test of high sensitivity for IgE-mediated allergy is that of the skin-prick test[11-13]. The allergic responses to the allergens in sensitised persons can be easily measured. Besides being used for identifying sensitised patients, this test is also used for detecting the presence of protein allergens in some latex products[12,14,15]. The protocol[14] involves the introduction of a small amount of the allergen into the skin, usually on the forearm, by first placing a drop of the extract on the skin, followed by lightly piercing through the drop with the tiny tip of a 1-mm sterile lancet with shoulder to prevent further penetration of the skin. The size of the wheal developed is measured 15 min after application. A negative control of the test solution (physiological saline) without any antigen and a positive control of histamine (histamine dihydrochloride 10 mg/ml) are always included in the test battery. The test solutions are prepared from non-prewashed latex or rubber samples cut into small pieces) at a concentration of 1g/5ml of physiological saline at room temperature for 15 min.

The test reactions or responses are evaluated in relation to the histamine wheal. Reaction size of twice that or more of the histamine control is a strong positive reaction and is

denoted as 4+, same size as that of histamine is 3+ (a clear positive), at least one-half of that of histamine is 2+ (a weak positive) Smaller wheals are not considered to be positive

Relationship between Extractable Proteins and Allergic Responses

Extractable protein contents of some commercially available medical gloves, and gloves produced under various processing conditions, were determined by the modified Lowry microassay[8] Extracts of these gloves were also prepared for the skin-prick test

Thirty-nine glove samples were examined and the prick test was carried out in five groups on a total of 59 patients who showed positive latex hypersensitivity in Finland. Results are as shown in *Tables 1* and *2*

It is apparent that gloves with higher extractable protein contents are associated with higher degrees of the allergic reaction in persons showing latex hypersensitivity Gloves with low extractable protein levels, on the other hand, tend to show comparatively low or negligible responses. This relationship is consistent with that suggested for the case of some latex films[10]. However, threshold EP level below which many latex sensitised persons are not affected, has not been established in this paper This requires a study involving a greater number of latex sensitised persons and EP from a larger pool of gloves Nevertheless, according to the present results, it would appear that EP content of approximately 0 4 mg/g and lower generally produces more than 60% negative responses *(Figure 1)* For even higher negative responses, the EP content should preferably be kept as low as possible, *e g.* 0 1mg/g and less

TABLE 1 EXTRACTABLE PROTEINS IN LATEX GLOVES AND THEIR ALLERGIC RESPONSE AS SHOWN BY THE SKIN-PRICK TEST

Latex gloves	EP (mg/g)	Allergic response %		
		ve	2+	3+/4+
A/1	0 702	0	8	92
A/2	0 686	0	0	100
A/3	0 655	0	23	77
A/4	0 647	0	30	70
A/5	0 638	0	20	80
B	0 695	14	72	14
C	0 689	14	72	14
D	0 644	15	31	54
E	0 479	20	20	60
F	0 451	0	57	43
Low-powder	0 106	62	38	0
Siliconised – A	0 103	63	25	12
Siliconised – B	0 065	62	38	0
Chlorinated	0 023	100	0	0

Allergic responses
(4+) – Strong positive reaction
(3+) – Clear positive reaction
(2+) – Weak positive reaction
(-ve) – No positive reaction

TABLE 2. EXTRACTABLE PROTEIN CONTENTS OF GLOVES PRODUCED UNDER DIFFERENT PROCESSING CONDITIONS AND THEIR ALLERGIC RESPONSES AS SHOWN BY THE SKIN-PRICK TEST

Latex gloves (Treatments)	EP (mg/g)	Allergic responses %		
		-ve	2+	3+/4+
Unleached	1.679	0	50	50
Unleached *	0.729	37	38	25
No WL, DL 50°C/1' *	0.394	70	23	7
WL 50°C/5', no DL	0.243	62	31	7
No WL, DL 50°C/3' *	0.201	62	13	25
No WL, DL 50°C/10' *	0.128	70	23	7
WL 70°C/5'	0.124	40	40	20
No WL, DL/RT 16 h	0.100	60	33	7
WL 50°C/1', DL 50°C/½' *	0.097	62	31	7
WL 50°C/5' no DL *	0.093	87	0	13
DC/PV: no WL, DL/RT 16 h	0.091	60	33	7
WL 50°C/2' water spray ½' *	0.085	76	12	12
WL 50°C/5', 150°C/10', DL 50°C/3' *	0.083	100	0	0
WL 50°C/5', 120°C/20', no DL *	0.074	100	0	0
WL 50°C/2', DL 50°C/½' *	0.073	88	12	0
RC/HA/PV: WL 50°C/2', no DL *	0.069	86	7	7
WL 50°C/5', no DL *	0.061	90	10	0
WL 50°C/5', 120°C/20', DL 50°C/3' *	0.050	88	12	0
WL 50°C/1', DL 50°C/5' *	0.044	88	12	0
Low protein latex: WL/1', DL/½' *	0.040	76	15	9
WL 50°C/5', DL 50°C/5' *	0.038	80	20	0
WL 50°C/5', DL 50°C/10' *	0.037	100	0	0
WL 50°C/5', DL 50°C/2' *	0.036	88	12	0
RC/HA/PV: WL 50°C/2', spray ½' *	0.028	100	0	0
WL 70°C/5', DL/16 h, chlorinated	<0.020	73	27	0

WL – wet-gel leaching
DL – dry-film leaching
RT – room temperature
*Gloves dipped in cornstarch slurry for 10 s after vulcanised/drying
RC/HA/PV – recentrifuged prevulcanised latex
Except for the 'low protein latex', all gloves were prepared from prevulcanised latex.
Unless otherwise stated, all gloves were subjected to drying/ vulcanisation at 100°C/30 min before dry-film leaching, if any.

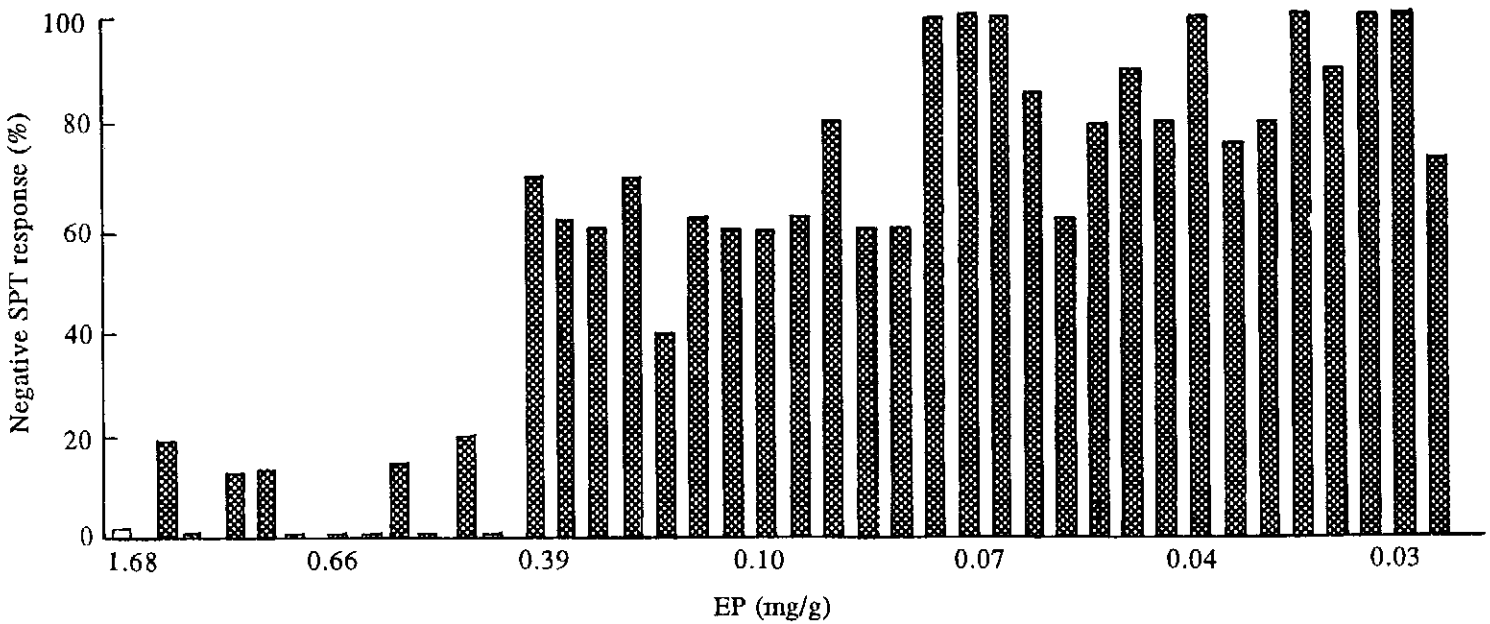

Figure 1. EP content of latex gloves and negative allergic response in latex hypersensitttive persons.

It may be noteworthy that while inadequate leaching of gloves is the most likely cause for the undesirable high residual extractable proteins, proper leaching under suitable processing conditions can reduce this EP to a level of low or negligible allergenicity.

NR DRY RUBBERS AND DRY RUBBER PRODUCTS

Extractable Protein Content in Dry Rubbers

The processing of *Hevea* latex into dry rubbers and dry rubber products is different from that of latex products. Instead of dipping, latex is usually converted into dry rubber by acid coagulation, followed by crumbling/ creping, washing in water, and drying at 100°–130°C. Contents of extractable proteins in these rubbers are expected to be relatively low, since the coagula are always subjected to extensive washing during processing. This is found to be so, as shown by the very low EP values obtained from various grades of dry rubber *(Table 3)*. The low levels as indicated are in fact, reaching the measurement limits of the method employed[8].

Allergic Responses and Extractable Proteins

To study the allergenicity of dry rubbers, protein extracts from 14 dry rubber samples of various grades and five different rubber products were prepared and tested for their allergic responses by the skin-prick test. The dry rubber samples included the raw rubbers, compound mixes (ACS 1) and vulcanisates (with ACS 1 mix and cured at 140°C for 40 min). Rubber products were those of cut threads, hot water bottle and diver's flippers. A total of 31 patients shown to be latex hypersensitive was clinically tested in three groups. Results are as shown in *Table 4*.

It can be seen that the extremely low EP levels in dry rubbers and dry rubber products demonstrated very little or no allergic response in latex hypersensitive patients in all cases. These negative observations are strongly substantiated by the positive reactions elicited in the same patients by extracts from a certain brand of latex gloves known for their allergenicity. Hence, the protein allergy problem of some latex dipped articles does not necessarily affect the NR rubber products.

TABLE 3. EXTRACTABLE PROTEIN LEVELS IN VARIOUS GRADES OF DRY NR RUBBER, AS MEASURED BY THE MODIFIED LOWRY MICROASSAY AND CALIBRATED AGAINST BOVINE SERUM ALBUMIN (BSA).

Dry rubber	No. of sources	Mean protein content (mg/g)(against BSA)
SMR CV	5	< 0.020
SMR L	6	< 0.020
SMR 5	1	< 0.020
SMR 10	5	< 0.020
SMR 20	5	< 0.020
RSS	2	< 0.020
Steam-coagulated	1	< 0.020
DPNR (normal)	1	0.022
DPNR(food grade)	1	< 0.020

TABLE 4. EXTRACTABLE PROTEINS AND ALLERGIC RESPONSES OF DRY RUBBERS AND DRY RUBBER PRODUCTS

NR dry rubber	EP (mg/g)	Allergic response by skin-prick test (%) - ve	2+	3+ / 4+
SMR CV/ raw	< 0.020	100	0	0
SMR CV/ compound mix	< 0.020	100	0	0
SMR CV/vulcanisate	< 0.020	100	0	0
SMR L/ raw	< 0.020	90	10	0
SMR L/ compound mix	< 0.020	100	0	0
SMR L/ vulcanisate	0.022	100	0	0
SMR 10/ vulcanisate	< 0.020	100	0	0
SMR 20/ raw	< 0.020	90	10	0
SMR 20/ compound mix	< 0.020	100	0	0
SMR 20/ vulcanisate	< 0.020	100	0	0
RSS / raw	< 0.020	88	0	12
RSS / vulcanisate	0.027	100	0	0
DPNR/normal grade/raw	0.022	90	10	0
DPNR/food grade/raw	< 0.020	100	0	0
Cut thread A	< 0.020	100	0	0
Cut thread B	0.029	90	10	0
Cut thread C	< 0.020	100	0	0
Hot water bottle	< 0.020	100	0	0
Diver's flippers	0.034	100	0	0
Latex glove*	0.647	0	30	70
Latex glove*	0.655	0	23	77
Latex glove*	0.686	0	0	100

DPNR – Deproteinised natural rubber, prepared by enzyme treatment of latex
*Latex gloves known to show positive responses
Allergic responses – see *Table 1*.

Consequently, the campaign against these products by some non-NR producers is therefore unwarranted. This is particularly so in the case of the cut threads, since they are generally covered by fabric thereby minimising any contact with the human skin. Furthermore, there are relatively fewer dry rubber products used in the healthcare sector where prevalence of latex hypersensitivity is often reported.

CONCLUSIONS

High contents of extractable proteins in latex medical gloves are generally associated with more positive allergic response in latex hypersensitive persons. Low extractable protein contents, on the other hand, tend to show weak or in some cases, no positive responses. No threshold levels have been established.

Dry rubbers and dry rubber products have extremely low extractable protein levels. When tested clinically these materials demonstrated very low or negligible allergenicity. Therefore, it may be concluded that these products are relatively little or not affected by the protein allergy problem encountered by some latex-dipped products.

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