

Proteins Eluted from Natural Rubber Crumb[†]

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The protein eluted from vulcanised natural and styrene butadiene rubber crumb samples has been subjected to total amino acid analysis and the total protein estimated. An antiserum raised to the NR crumb eluate reacted with the homologous immunogen and with polypeptides of the C-serum of fresh Hevea latex. The allergenicity of the natural crumb eluate has been compared to that of hevein in skin prick testing.

The possible sources of sensitisation to rubber latex proteins are unclear. One possibility is that the population may be frequently exposed to low doses of latex proteins as a result of inhalation of respirable particles of vulcanised rubber possibly present in urban air, originating from rubber tyre wear. The presence of such particles in particulate air samples derived from a rotary impaction air sampler stationed 7.4 m above ground level and 48 m from a moderately travelled road, has been demonstrated by Brock Williams *et al.*¹ An extract obtained from rubber tyre millings, the protein concentration of which was not specified, was found to inhibit the interaction of 6 out of 7 specimens of sera from latex sensitive persons reacting with a commercial preparation of latex allergens to a similar extent to that obtained with a latex glove protein extract containing 1 mg protein per ml. A further communication from the same group² claims the 'tentative' identification of a 50 kD polypeptide reacting with latex specific IgE in immunoblots of abraded tyre extracts.

In contrast to the above findings the *in vivo* tests of Yip *et al.*³ failed to demonstrate allergenicity of vulcanised rubber extracts in

skin prick testing of a panel of 31 latex sensitive patients. Whilst all of the patients responded to the different glove extracts tested none of the subjects responded to extracts obtained from samples of vulcanised rubber. There was however no indication of the protein levels in the extracts used save that those of the extracts of vulcanised rubber were apparently less than 4 µg/ml. Yip *et al.*³ also carried out RAST inhibition assays using a similar commercial latex allergen preparation to Brock Williams *et al.*¹, and with a calibrated pool of serum containing anti-latex IgE were unable to detect appreciable levels of allergen in any extract. Again the protein concentration in none of the tested samples was given.

More recently Miguel *et al.*⁴ have compared the extractable protein from latex gloves with that of dust from truck and radial car tyres. A pool of sera from latex allergic subjects was found to bind to specific peptides in immunoblots of electrophoretic separations of both truck and car tyre extracts.

With this conflict of evidence in mind it appeared desirable to establish the presence or

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otherwise of proteins associated with fragments of vulcanised rubber prior to any possible environmental survey of the presence of such respirable fragments in the atmosphere. To this end the eluates obtained from vulcanised rubber crumb have been analysed and tested for immunogenicity and antigenicity.

MATERIALS AND METHODS

Rubber crumb, vulcanised SBR and NR rubber was kindly provided by Dr A.D. Roberts of the Tun Abdul Razak Laboratory. Extracts were prepared by shaking the crumb (100 g) with phosphate buffered saline containing 0.01% merthiolate and 0.01% NaN_3 (400 ml) for 16 h at room temperature. The extract was filtered and concentrated to about 5 ml by pressure dialysis, centrifuged and freeze-dried. The freeze-dried material was reconstituted in 10 ml water and applied to a 10 ml *Sephadex G-25* column from which it was eluted with distilled water. The eluate was monitored by absorption at 280 nm and by assay using the Bradford protein assay⁵. Electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate SDS PAGE was carried out according to the procedure of Laemmli⁶. Immunoblots were obtained by the method of Towbin⁷. Total amino acid analyses were made on material hydrolysed in HCl for 24 h at 110°C. Antisera were obtained after 4 successive immunisations of each approximately 25 µg in Freund's adjuvant at fortnightly intervals.

RESULTS

Elution of the concentrated extracts from both NR and SBR rubber from *Sephadex G-25* columns showed the presence of high and low molecular weight components absorbing at 280 nm and both components gave a wavelength absorbance shift to 595 nm when

tested with Coomassie blue in the Bradford assay (*Figure 1*).

Analysis by SDS PAGE of extracts of both NR rubber showed the presence of a polydisperse range of polypeptides, the principle components having molecular weights of 58, 54, 43, 34 and 32 kDa. Amino acid analysis of the hydrolysate of the high molecular weight component of the extracts gave the results shown in *Figure 2*. From these results it was calculated that the eluted protein from the NR rubber crumb comprised approximately 0.23 p.p.m. vulcanisate and that of the SBR rubber 0.045 p.p.m. vulcanisate.

Immunisation of a rabbit with the NR extract gave an antiserum reacting with NR crumb polypeptides with approximate molecular weights of 100, 32, 29, 20 and 18 kDa and with those of C-serum with molecular weights of 100 (strongly) and 25 and 21 (weakly). The antiserum did not react with the proteins of the bottom (lutoid) fraction of latex

Skin prick testing of 6 selected latex allergic persons showed that whilst 5 gave a positive response to hevein (a major latex allergen), only one responded to the NR crumb extract (each at a concentration of 10 µg/ml).

DISCUSSION

It appears from the above results that whilst vulcanised crumb has associated protein, the level of elutable protein is approximately three orders of magnitude less than that usually found associated with latex goods, the SBR sample studied had a level of associated protein about one fifth of that of the NR crumb. These protein values obtained from total amino acid analysis were a thousandfold less than those reported by Miguel *et al.*⁴ using indirect methods of analysis.

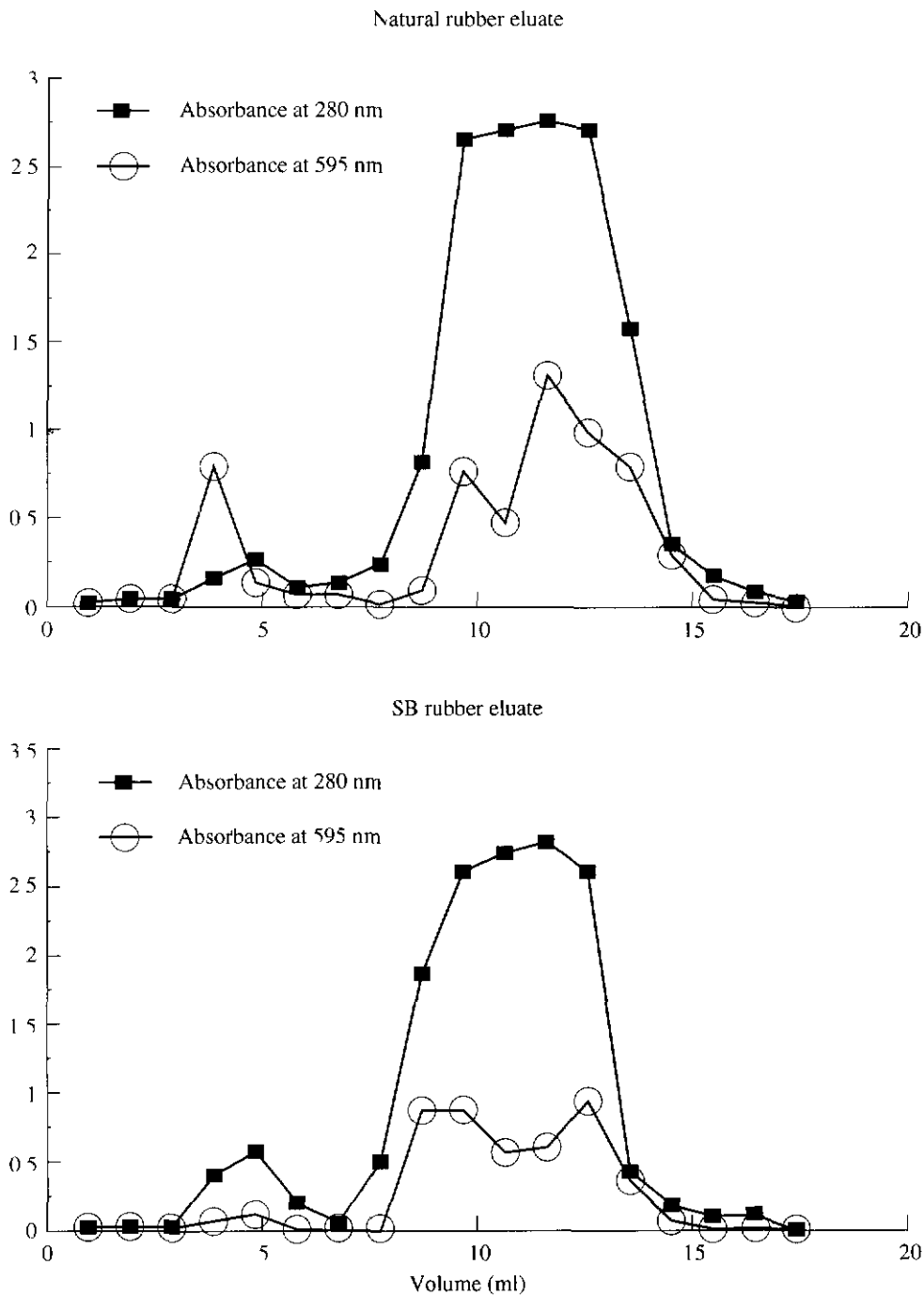


Figure 1 Elution of rubber eluates from Sephadex G 25 Absorbance at 595 nm indicates monitoring by the Bradford assay

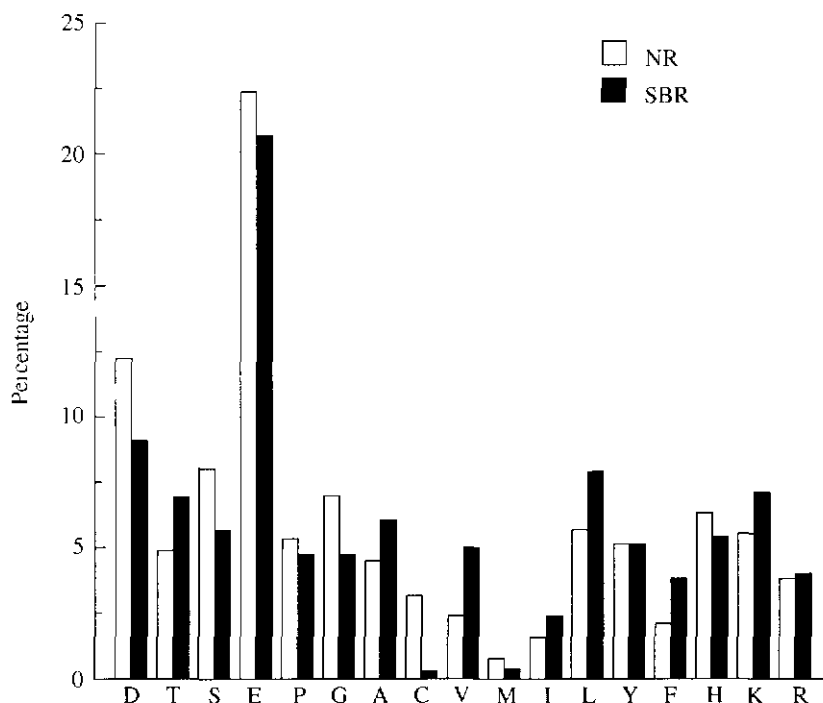


Figure 2. Amino acid analysis of rubber eluates.
The single letter code for amino acids is used

It is therefore concluded that whilst vulcanised NR crumb does have a trace of associated antigenic protein, it is far lower than that reported for tyre dust measured by indirect methods. Thus if the protein in vulcanised NR crumb approximates to that of tyre dust, the potential sensitisation risk should be considerably less than would appear from the studies of Brock Williams *et al.*¹ and Miguel *et al.*⁴. This is borne out to some extent by the sample of patients subjected to skin prick testing.

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