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Changes to NR Latex Proteins on Processing the Latex to its Products

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Fresh NR latex contained two major proteins, 14 kD and 24 kD, associated with rubber particles, and a variety of acidic and basic proteins of molecular weights less than 14 kD to greater than 100 kD in the B- and C-serum fractions Processing the latex to its products changed the composition of these proteins, more so with serum proteins than with rubber particle proteins Serum proteins most affected were the very acidic and basic proteins of molecular weight greater than 14 kD and isoelectric point (pI) in the pH range of 3 5 to 4 6 and 7 0 to 9 5, respectively The composition of these proteins was greatly influenced by the storage periods of high ammoniated (HA) latex concentrate, latex compounding ingredients and heating of the latex compound Thus HA latex concentrate of more than 3-months-old, HA latex concentrate compounded with ZnO and prevulcanised latex contained mainly acidic proteins of pI in pH range of 4 6 to 6 0 and molecular weights less than 14 kD A similar pattern was found in extractable proteins of NR examination gloves

Studies on NR latex proteins commenced way back in the 1950s The pace of research picked up after 1980s when it was established that soluble proteins extracted from latex products were linked to Type I hypersensitivity reaction in latex sensitised people[1–3] Following this, numerous reports[4–8] on proteins in fresh latex, ammoniated latex and latex products, particularly examination gloves appeared The focus was, however, on NR latex proteins that could bind to IgE antibodies which might result in an allergic reaction. Results from some of these studies showed that the protein composition changed on processing fresh latex to HA latex concentrate and to its products With the exception of the study by Hasma[6], most of these are isolated studies, with no definite intention of systematically giving a clear picture of the changes on the latex proteins in processing the latex to its products It is the objective of this study to add further information to the earlier work[6] besides presenting a clearer picture of the proteins in fresh NR latex and showing the effects of concentrating the latex, storing and compounding HA latex concentrate, and heating compounded latex The composition of proteins extracted from examination gloves was also examined This will enable latex researchers, producers and consumers to have a better understanding of the nature of proteins in NR latex and its products.

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MATERIALS AND METHODS

Isolation of NR Latex Proteins

Proteins from fresh latex. Fresh latex was centrifuged on a Beckman L8-70 ultracentrifuge at 19 000 r.p.m. using rotor 21 for an hour. Rubber particles (RP), clear C-serum and bottom fraction were isolated. RP were redispersed in 0.7% ammonia twice before a final dispersion in 2% sodium dodecyl sulphate (SDS). At each step, RP dispersion was filtered to remove any coagulum, stored overnight and finally ultracentrifuged to separate the RP from the extracts. The second ammonia extract and SDS extract were collected, dialysed against distilled water for 3 days and freeze-dried.

The bottom fraction was freeze-thawed three times, centrifuged at 10 000 r.p.m. using rotor 21 for 45 min before obtaining the clear B-serum.

Proteins from HA latex concentrate. Clonal HA latex concentrates were prepared in the laboratory by centrifuging about 0.5% ammoniated field latex in a De Laval LRH 410-70A centrifugal latex separator. Latex concentrates of about 60% dry rubber content was further ammoniated to 0.7%. Commercial latex concentrates of mixed clones preserved with ammonia and other secondary preservatives were obtained from a few local producers.

HA latex concentrates were ultracentrifuged to separate the RP from the serum fraction as above. The clear HA serum fraction was isolated, dialysed and freeze-dried.

Proteins from compounded and prevulcanised latex. HA latex concentrates were compounded with sulphur vulcanising ingredients according to the formulations tabulated in *Table 1*, stored overnight and ultracentrifuged to obtain the clear serum fraction. The compounded latex serum was dialysed and freeze-dried.

A portion of the compounded latex was matured overnight and prevulcanised at 70°C for 2 h. After overnight storage, the prevulcanised latex was ultracentrifuged and its clear serum collected, dialysed and freeze-dried.

Extractable proteins from examination gloves. Pieces of gloves weighing 10 g were extracted with 50 ml of 0.01 M phosphate buffered saline solution at 35°C for 3 h. The extracts were filtered through Whatman filter paper No.1, dialysed and freeze-dried. The dried sample was redissolved in 1 ml water.

Polyacrylamide Gel Electrophoresis

The B- and C-sera of fresh latex were diluted 1:4 (v/v) with a solubilising buffer while the ammonia and SDS lyophilised extracts of RP were solubilised in the same solubilising buffer to a concentration of 4 mg/ml. The mixture was heated at 95°C for 4 min before being analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) following the procedure outlined in Bio-Rad Instruction Manual[9]. The SDS-PAGE was run for 35 min at a constant voltage of 200 volts (Mini Protean 11 Cell; Bio-Rad, Richmond, Calif., USA).

The freeze-dried samples from serum of HA latex concentrate, compounded latex and prevulcanised latex and from glove extracts were solubilised in the same manner as above. The analysis by SDS-PAGE was, however, in

TABLE 1 COMPOUNDING FORMULATIONS

Compounding formulation	F_1	F_2	F_3
60% HA latex concentrate	100[a]	100[a]	100[a]
50% Sulphur	1 0	1.0	1 0
50% Zinc diethyl dithiocarbamate	0 6	0.6	0 6
50% Zinc oxide	–	0 4	0 4
20% Potassium laurate	–	–	0 2
10% Potassium hydroxide	–	–	0 2

[a] Values in p p h r

accordance with the method described by Schagger and Jagow[10]. This method gave a better separation of low molecular weight proteins of 3.5 kD to 17 kD.

Isoelectric focusing (IEF) was carried out according to the LKB Instruction Note 2217[11] on a 0.5 mm polyacrylamide gel containing ampholine carrier ampholyte pre-blended to pH 3.5 to pH 9.5, purchased from LKB (Bromma, Sweden). This technique was only used to analyse the water soluble proteins since the water insoluble ones could not be resolved well under the present experimental conditions About 20 µl of water soluble protein samples (4 mg/ml) were run at a constant power of 10 watts for 45 min with 1 M NaOH as cathode electrolyte and 1 M H_3PO_4 as anode electrolyte

The SDS-PAGE and IEF gels were fixed, stained and destained according to the respective instruction manuals[9–11].

RESULTS AND DISCUSSION

Proteins in Fresh Latex

Figure 1 shows a wide range of proteins from 3 major fractions of fresh latex; the RP, the B- and C-serum as analysed by the SDS-PAGE. As reported[12], the 14 kD and 24 kD proteins formed the two major RP proteins extracted by ammonia while the 14 kD protein, identified as the rubber elongation factor by Dennis and Light[13], was the main RP protein extracted by SDS The REF (*Hev b 1*) and the 24 kD protein (*Hev b 3*) have been shown to bind to the IgE antibodies of patients with *spina bifida* and latex allergy[14–16]

The B-serum clearly revealed protein bands of molecular weights less than 14, 14, 20, 22, 23, 30, 36 and 45 kD, in agreement with some of the B-serum proteins identified by Alenius[7]. The 20 kD prohevein has been demonstrated to exhibit high binding frequency to IgE antibodies of adult latex-allergic patients, suggesting it to be the major NR latex allergen[17] However, the main allergenic epitope of prohevein is located at its N-terminus, which represents the 4 7 kD hevein molecule[17]. This makes hevein an important latex allergen Hevein could be among those in the molecular weight region of less than 14 kD The allergenicity of 30, 36 and 45 kD proteins have also been demonstrated[17].

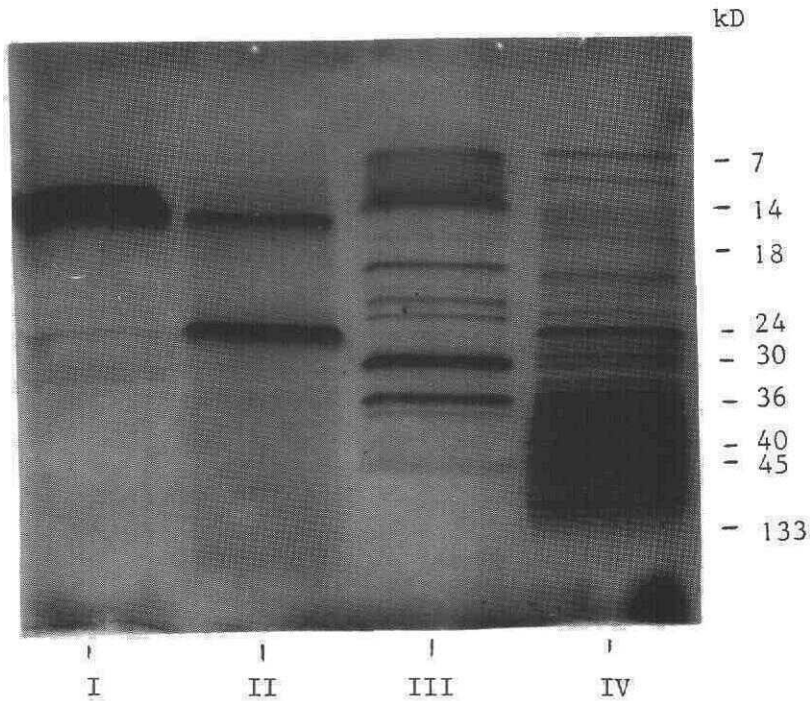

Figure 1. SDS-PAGE of fresh latex proteins.

I: SDS-extracted RP proteins
II: Ammonia-extracted RP proteins
III: B-serum proteins
IV: C-serum proteins

C-serum, however, contained a greater variety of proteins with molecular weights stretching from 7 kD to 133 kD. In fact a higher proportion of the C-serum proteins were of molecular weights greater than 36 kD, unlike the majority of the B-serum proteins which were of molecular weights less than 36 kD. The 27 kD C-serum protein has been found to be recognised characteristically by the IgE of latex-allergic children with *spina bifida* or other congenital anomalies and histories of multiple surgeries[18].

Analysis by IEF (*Figure 2*) showed that a high number of the ammonia-extractable RP proteins, the B- and C-serum proteins were acidic/anionic proteins with isoelectric points (pI) in the acidic pH regions of 3.5 to 6.0. The acidic proteins of the ammonia-extracted RP protein seemed to concentrate around pH 3.5

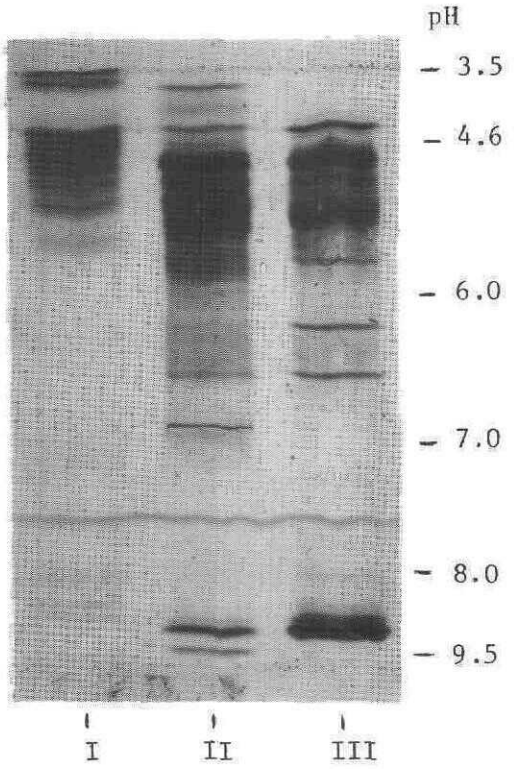

Figure 2. IEF of fresh latex proteins.

I: Ammonia-extracted RP proteins
II: C-serum proteins
III: B-serum proteins

to less than 6.0 while the B-serum proteins stretched from pH 4.6 to pH 6.8 and the C-serum proteins from pH 3.5 to pH 6.8. Protein bands were observed at pH 4.7 in B-serum and pH 4.55 in C-serum which could be hevein and α-globulin, respectively. However, based on the intensity of the two bands when compared to the rest, they did not represent the major B- and C-serum proteins of fresh latex. Hevein was reported to constitute 70% of the B-serum proteins while α-globulin was considered the highest concentrated protein in C-serum[19,20].

Unlike the acidic/anionic proteins, there were fewer number of basic/cationic proteins at pH 8

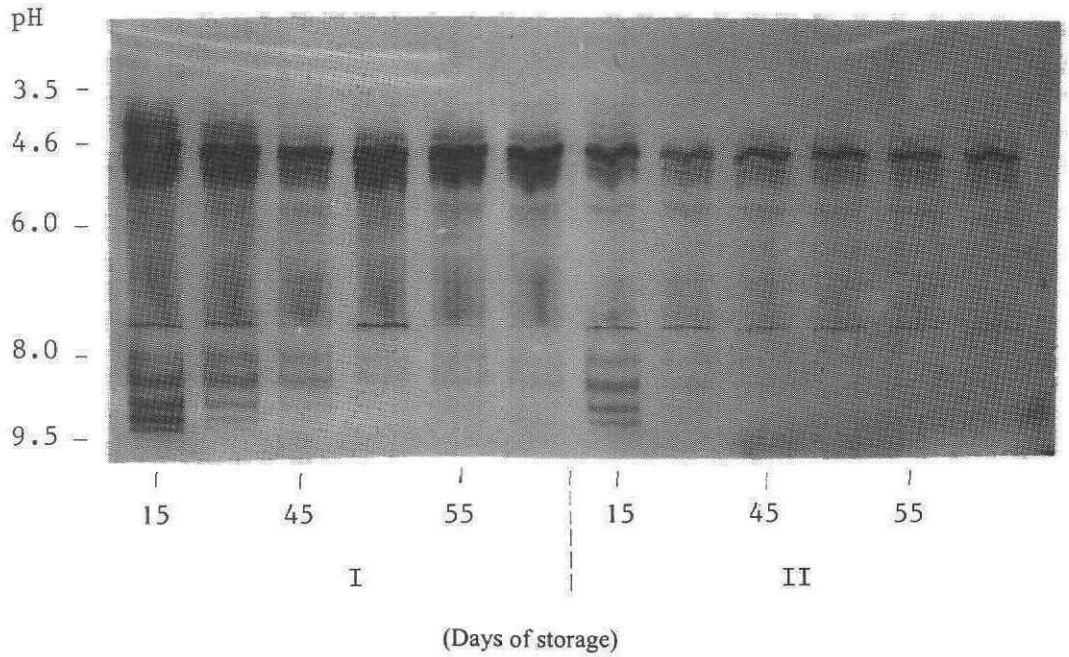

Figure 3. IEF of proteins from HA latex concentrate with (II) and without (I) secondary preservatives.

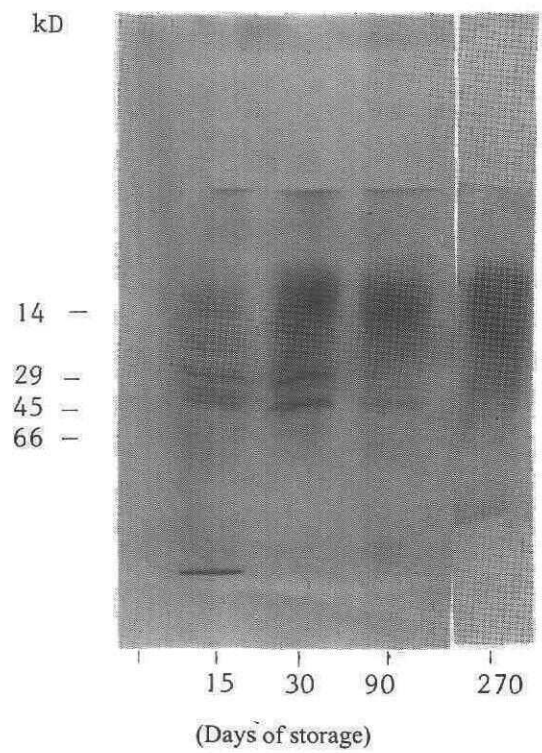

Figure 4. SDS-PAGE of proteins from HA latex concentrate.

to pH 9.5 in all the three latex fractions. Owing to the insolubility of the SDS-extractable RP protein in water, the protein was not analysed on the IEF However, the above analysis showed that fresh NR latex contained a wide variety of proteins with pI stretching from very acidic pH of 3.5 to basic pH of 9 5 and molecular weights of less than 14 kD to greater than 100 kD

Proteins in HA Latex Concentrate

The addition of ammonia and other preservatives such as tetramethyl thiuram disulphide-ZnO to field latex resulted in the bursting of the lutoids, releasing the B-serum content into the C-serum fraction Consequently ammoniated latex contains only two fractions; the RP and the serum fraction Centrifuging the latex to prepare latex 'concentrate resulted in not only the loss of proteins which are removed together with skim latex, but also changes to the composition of the proteins (*Figures 3* and *4*). These changes did not arise much from the RP proteins as similar proteins were extracted by ammonia and SDS from the RP of the latex concentrates of different storage times[12] The differences were, however, in the serum proteins. HA latex concentrate of up to 15 days old contained a number of acidic and basic proteins as shown in *Figure 3*. The composition of these proteins changed on prolonged storage of the latex The very acidic proteins of pI in the pH range of 3.5 to 4 6, and especially the basic ones greatly diminished after a month of storage, leaving the latex with mainly the acidic proteins of pI in the pH region of 4 6 to 6 0. The degradation rate was faster with the commercial HA latex concentrate which contained other secondary preservatives besides ammonia, than the laboratory prepared HA latex concentrate preserved with only ammonia However, after 2–3 months of storage, serum of all HA latex concentrates contained mainly acidic proteins with a prominent band around pH 4 7 which is also the pI for hevein.

Likewise, changes were also shown by SDS-PAGE (*Figure 4*) A number of high (>14 kD) and low (<14 kD) molecular weight proteins were present in the serum of freshly prepared to 15 days old HA latex concentrates After 15 days to a month of storage, the concentration of low molecular weight proteins in the latex concentrate increased. The level of high molecular weight proteins, however, diminised. On increasing the storage time further the amount of high molecular weight proteins became negligible such that older HA latex concentrate of more than 3-months-old contained only low molecular weight proteins of <14 kD.

Although latex concentrate serum did not contain the identified fresh latex allergenic proteins which are $\geq$ 14 kD, they could inhibit the binding of IgE to these proteins[21] This indicates that the serum proteins of latex concentrate contained epitopes/allergenic determinants of fresh latex allergens Thus degradation/hydrolysis of fresh latex proteins resulted in low molecular weight peptides with some of the allergenic determinants intact

Proteins in Compounded and Prevulcanised Latex Serum

Compounding the HA latex concentrate with sulphur, zinc diethyl dithiocarbamate (ZDEC), zinc oxide (ZnO), potassium hydroxide (KOH) and potassium laurate affected the composition of the serum proteins (*Figure 5*). The effects

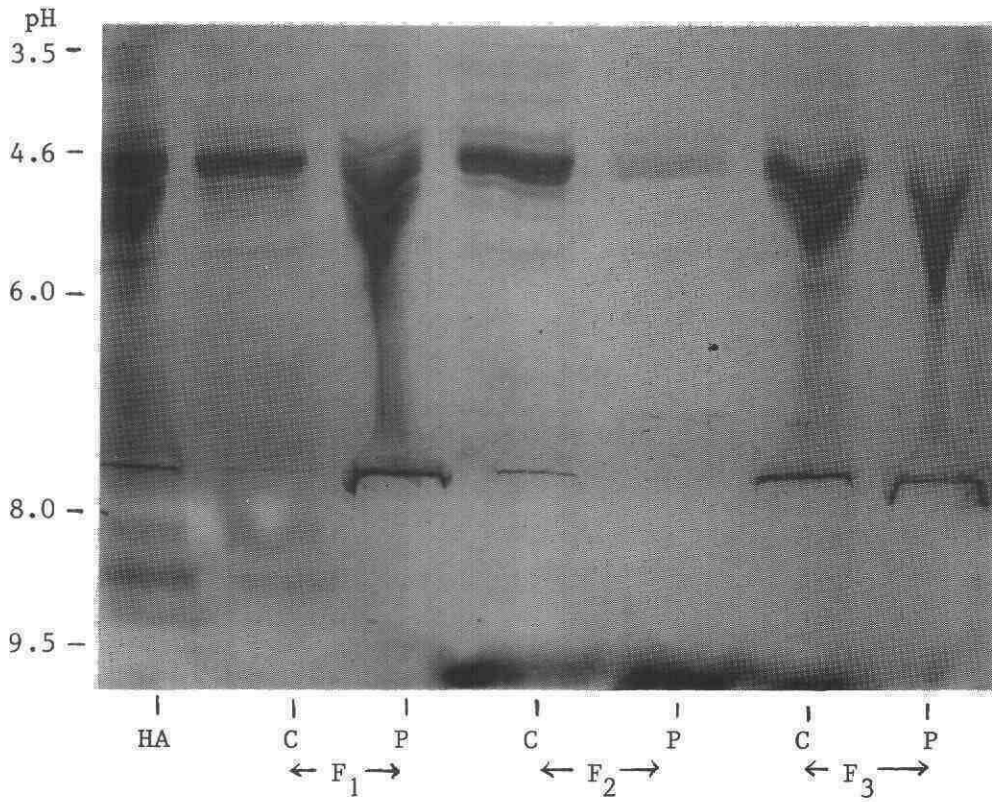

Figure 5. IEF of proteins from HA latex concentrate (HA), compounded latex (C) and prevulcanised latex (P) of different formulations (F).

were mostly on the basic proteins brought about by the presence of ZnO. When the latex concentrate was compounded with formulation 1 (*Table 1*) (in the absence of ZnO), the basic proteins were still present though at a lower concentration than originally present in the starting material of a month-old HA latex concentrate. These proteins were, however, rendered undetectable on incorporating ZnO in the presence or absence of KOH and potassium laurate. Heavy metals such as Zn are known to complex with proteins rendering it insoluble. Heating the compounded latex at 70°C for 2 h with or without ZnO, also resulted in the basic proteins being undetected. Furthermore, the very acidic proteins of pI in the pH range of 3.5 to 4.6 which were present in the serum of the compounded latex were denatured on heating the latex.

The difference in the serum proteins of the compounded latex as shown by the IEF (*Figure 5*) was not evident on SDS-PAGE (*Figure 6*). The serum of compounded latex of

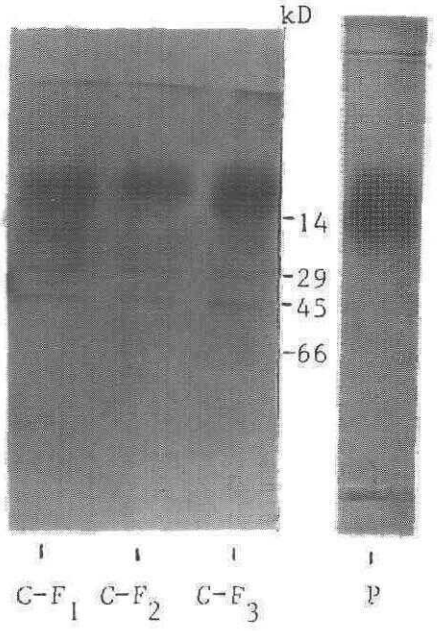

Figure 6. SDS-PAGE of proteins from compounded (C) prevulcanised (P) latex of different formulations (F).

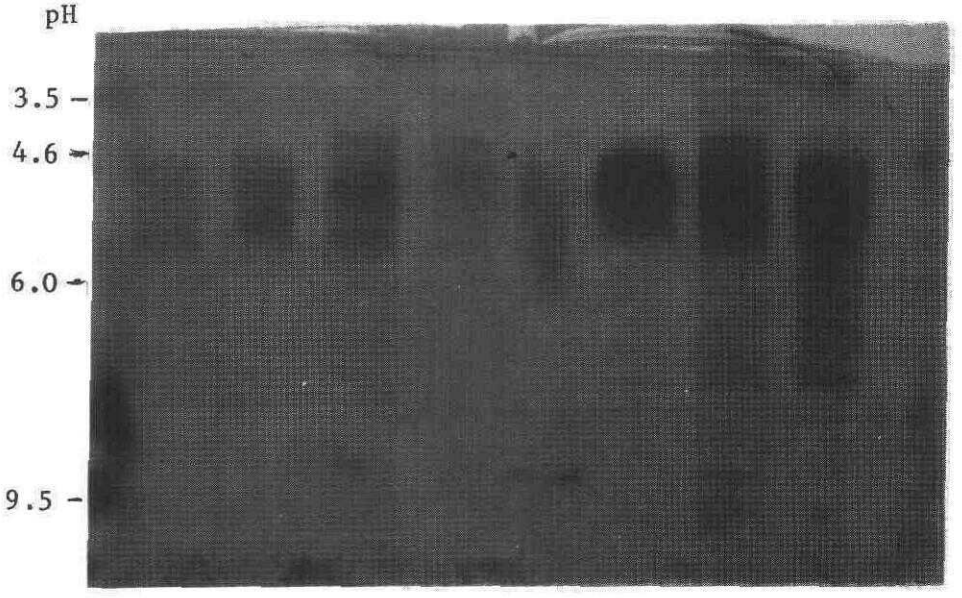

Figure 7. IEF of extractable proteins from commercial examination gloves.

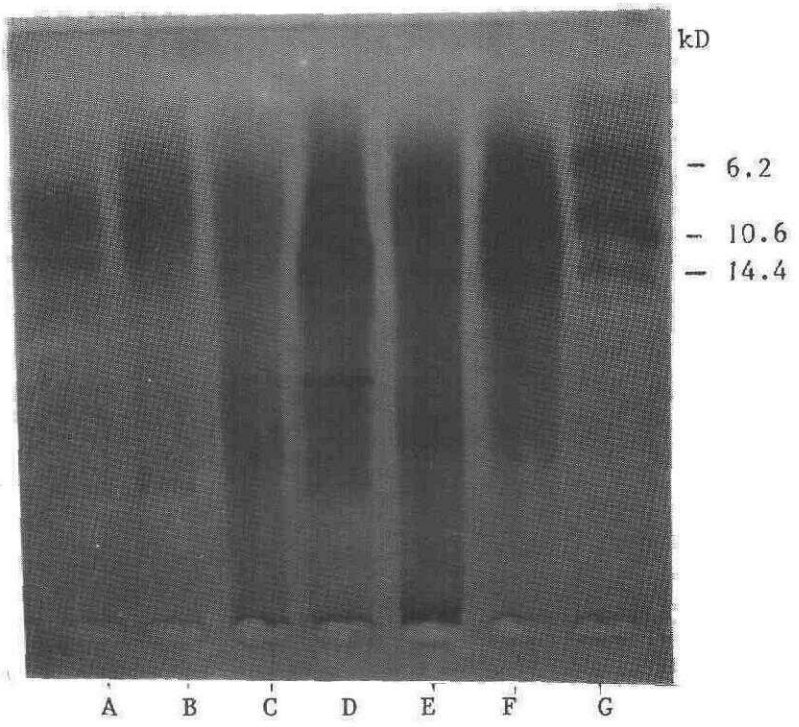

Figure 8. SDS-PAGE of extractable proteins from commercial examination gloves.

A-F: Extractable proteins from gloves
G: Low molecular weight markers

all the three formulations showed similar composition of proteins of molecular weights of <14, 29 and 45 kD. The prevulcanised latex, however, contained only the low molecular weight proteins of <14 kD. All the high molecular proteins of 29 kD and 45 kD could have been degraded on heating the HA latex concentrate at 70°C for 2 h. These proteins could include the very acidic proteins of pI in the pH range of 3.5 to 4.6 which were present in the three compounded latices but not in the prevulcanised latex. Consequently prevulcanised latex contained mainly acidic proteins (pH 4.6–pH 6.0) of molecular weight <14 kD.

Proteins Extractable from NR Examination Gloves

The IEF pattern of extractable proteins (EP) from the examination gloves (*Figure 7*) resembled more to that of the prevulcanised latex serum proteins, which formed the starting materials for most of NR latex gloves. The EP of about 20 gloves examined were mainly acidic proteins of pI in the pH range of 4.6 to 6.0. Basic proteins were only detected in one of the twenty gloves examined. This particular glove also contained a detectable level of high molecular weight proteins of greater than 14 kD besides a much higher concentration of low

molecular weight proteins of 6.2 kD to 14 kD (*Figure 8*). In fact the low molecular weight proteins of 6.2 kD to 14 kD formed the major proteins extracted from examination gloves (*Figure 8*). It could not be ruled out that hevein is not present in gloves extracts, as the present technique could not detect very low molecular weight peptides such as hevein.

CONCLUSION

Fresh latex contained a variety of acidic/ anionic and basic/cationic proteins with pI in the pH regions of 3.5 to 9.5 and molecular weights of less than 14 kD to greater than 100 kD. Adding ammonia and centrifuging the latex to HA latex concentrate resulted in a loss to the amount of proteins and changes to the composition of the proteins, particularly the serum proteins. The serum proteins most affected were the very acidic (pH 3.5–4.6) and basic (pH 8.0–9.5) proteins of molecular weights greater than 14 kD. The level of these proteins diminished on increasing the storage periods of the latex such that HA latex concentrate of more than 2–3 months old contained mainly acidic proteins (pH 4.6–6.0) of molecular weights less than 14 kD. Compounding the HA latex of less than a month old with sulphur vulcanising ingredients also affected the basic serum proteins. The effect was especially evident in the presence of ZnO but not in the presence of other normal sulphur vulcanising ingredients. Heating the compounded latex with or without ZnO, however, rendered the basic as well as the very acidic proteins undetectable. This explains the observation that prevulcanised latex comprises only acidic proteins of pI in the pH range of 4.6 to 6.0 and molecular weights of less than 14 kD. Similar protein composition was found in the extractable proteins of 20 brands of NR latex examination gloves.

Although gloves' extracts and sera of latex concentrate, compounded latex and prevulcanised latex do not contain the identified allergenic proteins of fresh latex, it is not unlikely that their peptides may still contain the relevant epitopes to enable them to elicit allergic reaction.

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