Introduction

Among plants which can produce latex, only the latex from *Hevea brasiliensis* has been exploited for more than 100 years. *Hevea* latex is found in latex vessels localized in the cortex, especially in the layer 2 to 3 mm thick nearest to the cambium. Like all plant materials, latex contains growth-related substances such as proteins, carbohydrates and other organic and inorganic constituents. The rubber hydrocarbon particles (the elastic component sought in all natural rubber products) comprise 25% to 45% of the latex system. The non-rubber substances constitute only a small percentage of the latex system.

Thailand is the world's largest producer and exporter of natural rubber (NR) since 1991. In 1995, a total rubber production of Thailand was about 1.81 million tons or about 30.6% of the world rubber production. About 90% of the rubber production was exported in the form of ribbed smoked sheet (67%), block rubber (19%), latex concentrate (10%) and a relatively small quantity of rubber air-dried sheet, crepe and skim rubber (4%). It is estimated that the rubber production in 2003 will be about 2.36 million tons which would correspond with an annual increasing rate of 1.3% (RRIT, 2002).

The consumption of raw rubber within Thailand is relatively quite low or less than 10% of the total production. The main rubber products of Thailand were tires and tubes, gloves, rubber bands and elastic. Of these main product sectors, the rubber glove industry has been growing fastest. Thailand is now the second high quality rubber glove producer and exporter with an export value of

13,000 million Baht in 1998, and may soon be the top latex glove producer/exporter (RRIT, 2002).

The recent emergence of latex protein allergy (type1 hypersensitivity), associated with the use of medical gloves and some other products, is of great concern to clinical professional and the manufactures. Prevalence has been reported to be less that 1% of the general population (Turjanmaa, 1987; Liss and Sussman, 1999), although it appears to be higher among selected risk group, such as employees in the health care sector. Research and development projects focusing on product improvement have been undertaken by latex glove manufacturing countries throughout the world to address the problem.

At present, the proteins in NR latex and its products were well known and some of them was identified as latex allergens. However, the origin background of the proteins remaining in latex glove is still unclear. This may be the limitation of the previous study that focusing on only the three fractions of fresh latex (rubber particles, B- and C-sera) as the source of proteins in NR latex. In this thesis, we proposed to investigate possible involvement of the bottom fraction membrane (BFM) as the another causative source contributed to latex allergy.

Literature Review

1. Natural Rubber Latex (NRL)

1.1 Introduction

There are many plants capable of producing latex; they belong to several different families but are mainly of the Dicoteledons (Metcalfe,1967). Latex is the fluid, generally milky in appearance, which flows from these plants after the slightest wound; it is produced and then stored, rarely, in parenchyma cells or more frequently in the tube structure known as laticifers

Of some 12,500 species of laticiferous plants, approximately 7,000 produced polyisoprene. In most case the polyisoprene is with mixed resin, making the latex difficult to use when the content of the latter is high. Finally only the limited number of rubber-producing plants can be exploited and only a few species are cultivated and have economic important (Bonner and Galstone, 1943). Among them, *Hevea brasiliensis* was soon revealed to be the best rubber producer. This tree species, which grow in the hot humid intertropical regions, is exploited by tapping the bark. *Hevea* possesses articulated laticifers in the barks, although it is thought that laticifers are also capable of apical intrusive growth in the cotyledons, inner seed coats, and in young leaves (Bobilioff, 1923). Laticifers form from procambial cells in young plantlets; they are found in the primary phoem in shoots, roots, and the veins of young leaves, and later in flowers and fruits. As soon as the cambium has formed it produced a special laticiferous system in the secondary phloem. Articurated, anastomosing laticiferous vessel from successive vertical networks called rings or

mantles. In *Hevea*, these secondary laticiferous vessel of the trunk are exploited by tapping the bark. The tree releases a large amount of latex at each tapping and can be exploited for more than a decade.

1.2 Fresh Hevea Latex

1.2.1 Major Constituents

The *Hevea* latex, as it flows out of the tree, is a complex cytoplasm containing a suspension of rubber and non-rubber particles in an aqueous medium (Southorn,1961 and Archer *et al.*, 1969). Using high speed centrifugation (59,000g), Cook and Sekhar (1953) separated latex into four fractions. There were: an upper white fraction of rubber cream, an orange or yellow layer containing Frey-Wyssling complexes, a colourless serum named C-serum and a greynish yellow gelatinous sediment the "bottom fraction" containing mainly of lutoids (Dikenson,1969 and Southorn,1966).

Moir (1959) using differential staining and high speed centrifugation techniques, showed that the sedimentable material in latex did not consist wholly of one species of particle. By treating the latex with trace amounts of Janus Green B or neutral red before centrifugation, he obtained eleven zones (Fig.1). Zone 1 correspond to the "top whitish fraction" of Cook and Sekhar (1953) which consists mainly of hydrocarbon particles. Zone 2 was a much smaller, translucent layer situated under the lowest portion of Zone 1. Zone 3 was a suspension of rubber particles in the serum. Zone 4 was the yellow and orange layer of Cook and Sekhar (1953). The aqueous Zone 5 correspond to C-serum and Zones 6-11 together were broadly equivalent to the "bottom fraction".

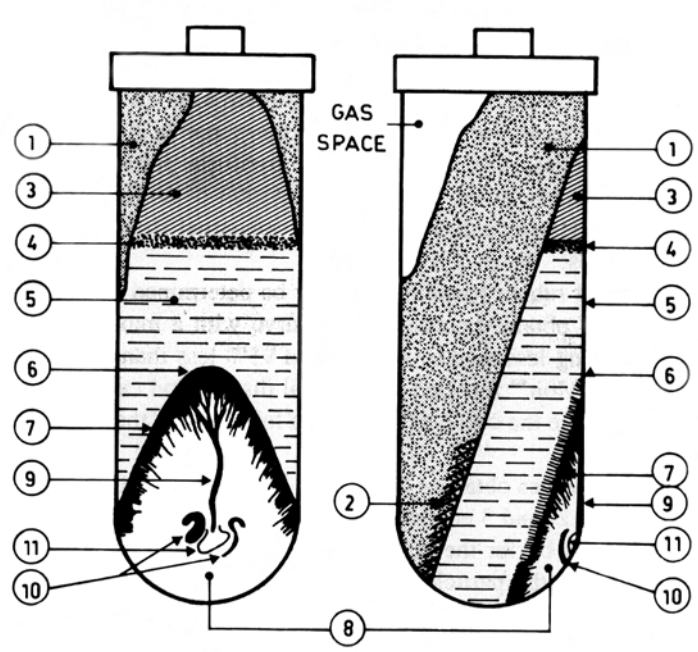

Fig. 1 Separation of fresh latex by ultracentrifugation (53,620 g max X 40 min). Fraction 1-3 correspond to white rubber phase. Fraction 4 is a yellow orange layer constituted by Frey-Wyssling particles. Fraction 5 is an almost clear serum (C-serum) corresponding to the latex cytosol. Fraction 6 to 11 constitute the "bottom fraction" in which highest in quantity is the lutoid fraction (Moir, 1959).

Rubber particles

The rubber particles usually have a size ranging from 50 A° to about 30,000 A° (3 μm), although extreme cases having 5 or 6 μm are also found. There are spherical bodies in young trees but in mature trees the particles are large, often having a pear shape. The shape in certain cases seems to be a clonal character. Pear shape is reported to be very frequent in clones such as Tjir 1 and PR 107 (Southorn, 1961).

A rubber particle of average size, about 1,000 A°, contains hundreds of molecules of the hydrocarbon and is surrounded by a surface film of proteins and lipids. The rubber particles are also associated with triglycerides, sterols, sterol esters, tocotrienols and other lipids. Dupont *et al.* (1976) have confirmed the presence of phosphotidylcholine and small amount of phosphatidyl ethanolamine in the lipids associated with rubber particles. The protein envelope of rubber particles is visible in sections of osmium stained rubber particles and is approximately 100 A° thick (Fig. 2) (Andrews and Dickenson, 1961). The envelope carries a negative charge and confers colloidal stability to the rubber particles.

According to Dickenson (1969) there are rubber particles with variously stained regions. An osmiophilic region surrounded by a weakly stained periphery attributed to lack of uniformity when rubber particles are deposited on existing particles during biosynthesis. He has also suggested that the inner particulate inclusion, having 50-80 A° thickness, might be molecules of rubber of molecular weight about 100,000.

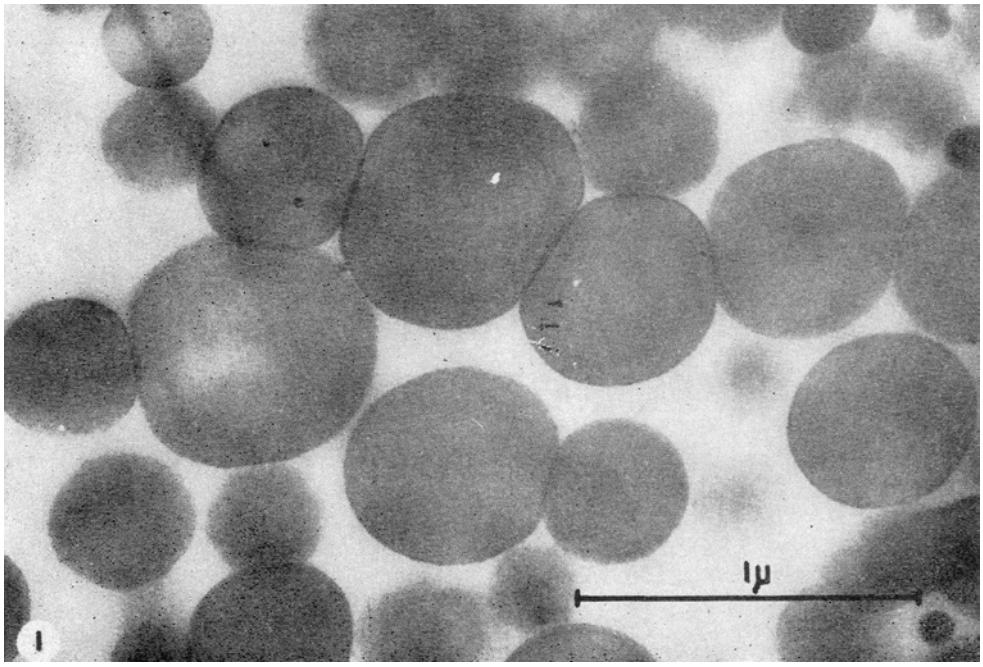

Fig. 2 Rubber particles in ultra-thin section showing, in some, the thin surrounding layer (Andrews and Dickenson, 1961).

Lutoids

Lutoids form the next major component of *Hevea* latex. They are membrane-bound bodies and mostly larger in size than the rubber particles. They are 2-5 μm in diameter bounded by a unit membrane of about 80 A° thickness (Fig. 3) (Dickenson, 1965; Gomez and Moir, 1979). It was Wiresum (1957) who first suggested that the lutoids behave like vacuoles due to stainability with neutral red. Though controversy existed in this regard, the work of Ribaillier *et al.* (1971) provided evidence for the vacuolar properties of lutoids.

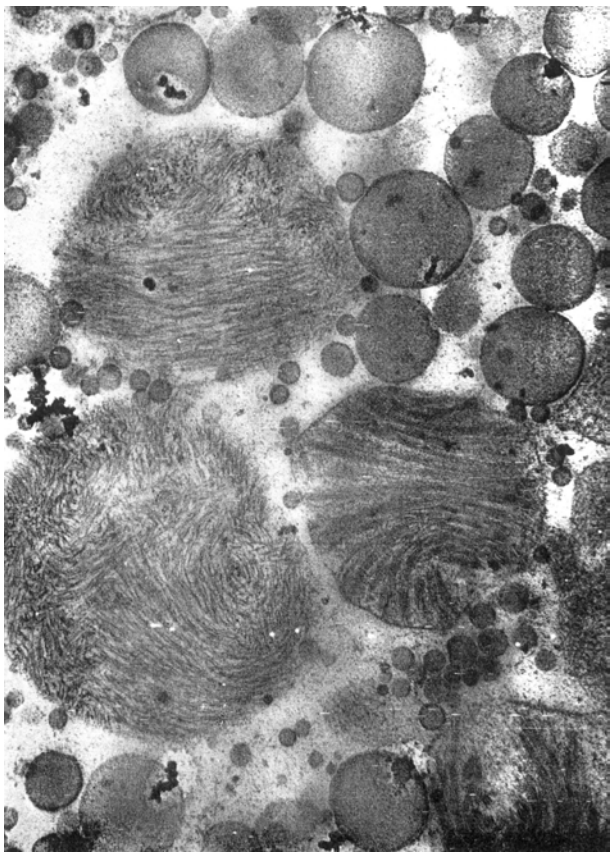

Fig. 3 Lutoid particles of two apparent morphological types in a young latex vessel of *Hevea brasiliensis* (Dickenson, 1965).

The content of lutoids (B-serum) has a very rapid flocculating action on aqueous suspension of rubber particles in lalex, resulting in the formation of microflocs (Southorn and Edwin, 1968). This activity is appearently moderated by the ambient C-serum and is much reduced if B-serum is boiled. Southorn and Yip (1968) demonstrated that this fast initial flocculating action of B-serum is an electrostatic one involving the interaction between the cationic contents of B-serum and the anionic rubber particle surface.

By phase contrast microscopy and application of suitable staining procedure the structure of lutoid particles have been studied in detail. Mainly two types of fibrillar structures (Fig. 4) have been described. The first type, known as microfibrils, are characteristics of latex vessels in young tissues (Dickenson, 1965, 1969; Audly, 1965, 1966). As seen by phase contrast microscopy of tapped latex from young tissue, the micofibrils are freely suspended in the fluid content of the lutoid B-serum. The microfibrils are seen usually as grouped together in bundles. Each bundle has a diameter of 450-500 A°. Individual microfibrils are several micron long and 70-80 A° in diameter. The microfibrils can be isolated from the sediments of latex from young tissues which on negative staining with phosphotungstic acid shows further details. Each microfibril is a tightly coiled continuous helix with hollow axis. The diameter of the helix is about 125 A° and that of hollow axis 30 A°. The microfibrils consist of an acidic protein while nucleic acid seem to be absent. Microfibrils however are not present in tissue or latex collected from the mature bark. It is believed that they disintegrated as the particles mature or else the young lutoids containing microfibrils themselves disintegrate as the tissue age and are replaced by a population of lutoids without microfibrils. However, the microfibrils do not seem to have vital role in rubber biosynthesis.

The second type of fibrilllar structures, observed in lutoids of latex from mature bark of stimulated trees, are known as ‘microhelices’, so named (Gomez and Yip, 1975) because of their spring like shape. These structures were first observed by Dickenson (1965, 1969). They are occasionally found in unstimulated trees and their number increases on dilution. However,

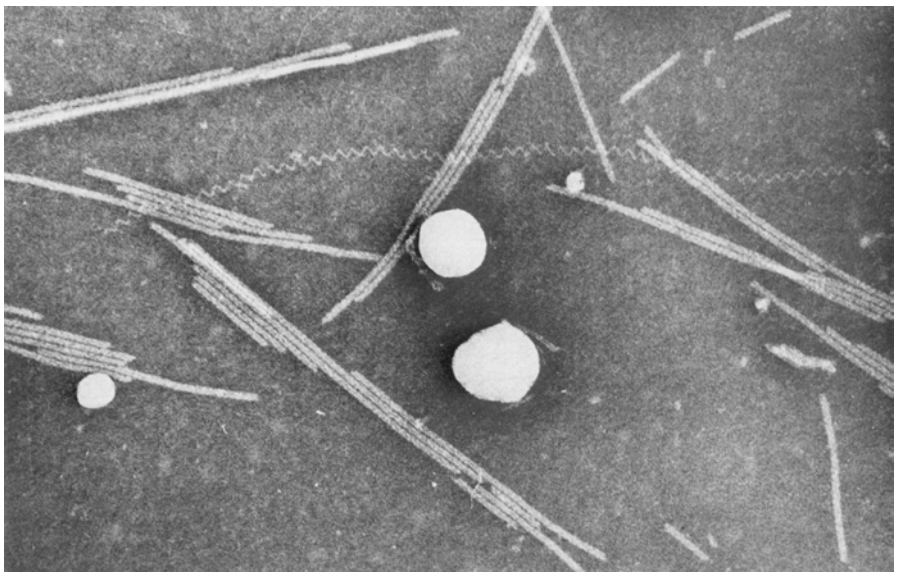

Fig. 4 Isolated microfibrils and one microhelix from latex of green stem.

microhelices are reported to be more frequent in lutoids of tapped latex than *in situ* latex (Southorn and Edwin, 1968; Gomez and Yip, 1975) and are occasionally observed in latex collected from young tissue also.

As reviewed by Gomez and Moir (1979) the microhelices are approximately 1 μm in length with a diameter of 200 A°, having a fibre width of about 50 A° and an open hollow helix having a 300 A° wide pitch. Dickenson (1965) suggested the formation of microhelices from microfibrils but this has been questioned by Gomez and Yip (1975).

A third type of lutoid inclusion – minute spherical particles in Brownian movement – was observed by Schoon and Phoa (1956). Later Southorn (1960, 1961) found such particles in large number in the botttom

fraction of ultracentrifuged latex of long rested trees and this was confirmed by Dickenson (1969). The role of such particles in latex is unknown.

Frey-Wyssling complexes

Yellow globules, in clusters in tapped latex were first noted by Frey- Wyssling (1929). The existence of such particles in groups, associated with a vacuolar body was observed by Southorn (1969) in phase contrast microscopy. He found that the individual particles are covered by a membrane, this was confirmed by electron microscopy. Dickenson (1969) named these particles, enclosed as a single structure, as Frey-Wyssling complexes.

The Frey-Wyssling complexes are more or less spherical in shape in a size range of 3-6 μm (diameter) and are bounded by a double membrane (Fig. 5). Within the membrane there are two types of particles – large osmiophilic globules in variable numbers and a system of rope-like tubules of about 750 A° diameter, usually embeded in a membrane bound matrix of osmiophilic nature.The complex structure of Frey-Wyssling complexes has been elaborated by Dickenson (1969) who described a series of concentric lamellae of the double unit membrane and the system of tubules and also highly folded invaginations of the inner membrane.

The Frey-Wyssling complexes are considered to have vital role in metabolic activities. Though Dickenson (1969) opined that these structures may be possible sites of rubber biosynthesis, the double membrane and presence of carotene and polyphenoloxidase in the Frey-Wyssling complexes led to a tentative suggestion that it is a type of plastid.

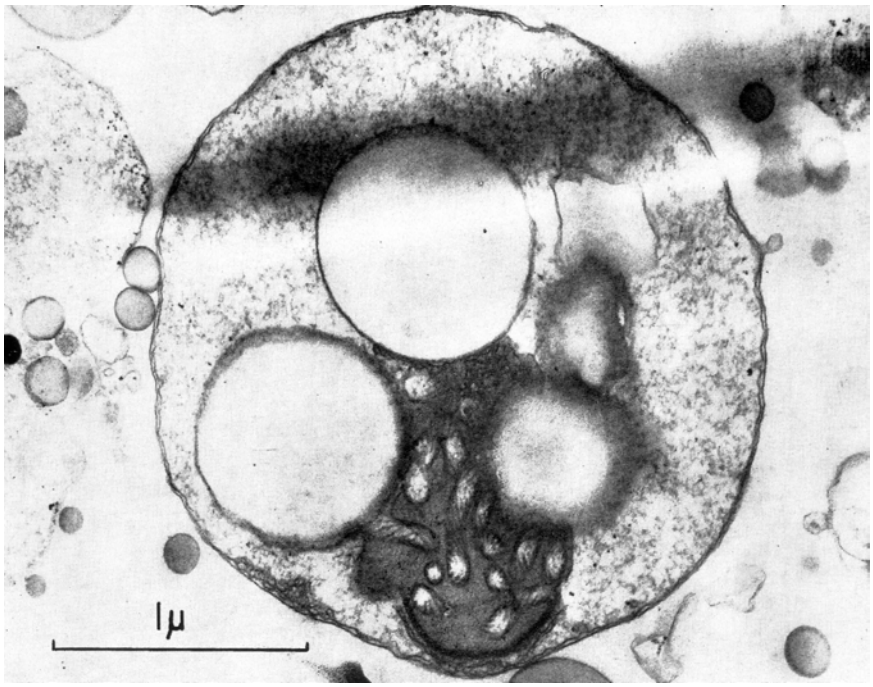

Fig. 5 A Frey-Wyssling complex showing the double membrane envelope; lipid inclusions; one system of tubules with interior spiral structures all contained in a membrane-bound osmiophilic matrix, with associated lamellae (Dickenson, 1969).

1.2.2 Organic Non-rubber Constituents

Proteins

The total protein content in latex has been estimated to be about 1% (Archer and McMullen, 1961; Archer *et al.*, 1963b; Tata,1980a). However, discrepancies in the distribution of the proteins between the major phases of latex exist. Archer and McMullen (1961) reported that 20% of the total proteins was absorbed on the rubber surface, 66% in the C-serum and 14% in the bottom faction. Later, reports variously described the distribution as 20%,

60% and 20% (Archer *et al.*, 1963b) and 27.2%, 47.5% and 25.3% (Tata, 1980a) for the rubber phase, C-serum and bottom fraction respectively.

Proteins on the surface of the rubber particle: The existence of proteins in association with phospholipids on the surface of rubber particles was recognized as early as 1953 by Bowler (1953). He attributed that this protein-phospholipid layer imparted a net negative charge to the rubber particle, thereby contributing to the colloidal stability of these particles. By measuring the iso-electric points of various latex samples, he concluded that there was more than one protein adsorbed on the rubber surface and that the relative proportion of the adsorbed proteins varied with clones.

Apart from the estimation that the protein adsorbed on rubber surface accounted for about 1% of the weight of rubber (Cockbain and Philpott, 1963). The major protein on the rubber surface has been shown to be negatively charged and has a molecular weight of approx. 65,000 (RRIM, 1982). It migrates towards the anode at a higher rate than the major C-serum protein α-globulin and contrary to the earlier suggestion, is therefore not identical with the latter.

Of the numerous enzymes reported in *Hevea* latex, only two have been found to be associated with the rubber surface. These isopentenyl pyrophosphate polymerase (Lynen, 1967; Archer *et al.*1963a) and rubber transferase (Lynen, 1967; Archer *et al.* 1963a; Archer and Cockbain, 1969; McMullen and McSweeney, 1966; Archer *et al.* 1966). Their presence on the rubber surface is not surprising, since they are involved in rubber biosynthesis.

Proteins in the C-serum: Nearly half of the enzymes examined in *Hevea* latex appeared to be located in the C-serum of latex. These include

enzymes for the glycolytic pathway (Bealing, 1969; d'Auzac and Jacob, 1969) as well as many of the enzymes for rubber biosynthesis (Archer and Audley, 1967). Twenty-seven enzymes were separated by electrophoresis by Jacob and co-workers, of which, seventeen were shown to exist in multiple forms (Jacob *et al.*, 1978).

The first protein to be isolated from *Hevea* latex was from C-serum. It was named α-globulin by Archer and Cockbain (1955). This protein is the major protein component of C-serum. It is readily adsorbed at a water-air or oil-water interface with a resulting fall in the interfacial tension. This led to the suggestion that α-globulin was one of the proteins on the surface of rubber particles and that it contributed to the colloidal stability of fresh latex (Archer and Cockbain, 1955). However, as mentioned earlier, α-globulin was later found not to be present on the surface of the rubber particles (RRIM, 1982).

With the introduction of more sensitive techniques, further discoveries on proteins of C-serum were made. Using starch gel electrophoresis, Tata and Moir (1964) reported the presence of twenty-two protein bands in C-serum. Seventeen of these were anionic at pH 8.2, whilst five were cationic and existed much concentrations. A comparative study on the proteins in the C-sera from four clones viz. RRIM 501, GT 1, Tjir 1 and Pil A44, revealed very little differences between their general electrophoretic patterns (RRIM, 1963). There was also no significant difference in the proteins with seasonal variation within a single clone. Later, the list of proteins in C-serum was enlarged to twenty-four (Tata and Edwin, 1970) using the same starch gel electrophoretic technique.

Using polyacrylamide gel electrophoresis, Yeang *et al.* (1977) reported 26 protein bands from C-serum at alkaline pH and 15 bands at acid pH.

Proteins in the bottom fraction: Proteins in the bottom fraction are essentially studied as the soluble proteins in B-serum. These have been examined with various techniques, including paper electrophoresis (Moir and Tata, (1960), starch gel electrophoresis (Tata, 1975; Tata and Edwin, 1969) and polyacrylamide gel electrophoresis (Yeang *et al.*,1977). Irrespective of the techniques used, the proteins of B-serum were found to be marked different from those C-serum. Upon electrophoresis (Audley, 1965; Karunakaran *et al.*, 1961; Moir and Tata, 1960), the B-serum proteins were usually separated into two major protein bands at the extreme anionic and cationic ends, with minor bands in between (Fig. 6).

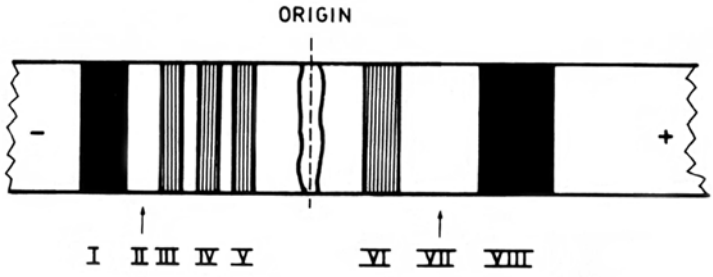

Fig. 6 Paper electrophoresis of dialysed proteins from lutoid fraction. The bands II and VII were not always visible. The anionic protein VI and the cationic proteins III, IV and V should be major constituents of microhelices. The principle cationic protein I is HEVAMINE which in fact is the lysozyme. The most important anionic protein VIII is HEVEIN (Moir and Tata, 1960).

Hevein: The major protein in B-serum is hevein, which accounts for about 70% of the water soluble proteins in the bottom fraction (Archer *et al*., 1969). Hevein is a low molecular weight anionic protein (approximate 5,000 daltons) with a higher (5%) sulphur content (Tata, 1975; Archer, 1960; Tata, 1976). All the sulphur in hevein exists as eight disulphide (S-S) bridge of cystine (Archer, 1960; Tata, 1976). Because of its low molecular weight and number of S-S bridge, hevein is heat stable, and is not precipitated by the common reagents for precipitating proteins eg. trichloroacetic acid (Tata, 1975; Tata, 1976). The molecular weight of hevein was first estimated to be about 10,000 ± 500 daltons by Archer (1960). Subsequent analysis showed that earlier preparations (Archer, 1960; Karunakaran *et al.*, 1961) of hevein were mixtures containing hevein, traces of esterase and a protein with slighly less anionic mobility than hevein, termed pseudo-hevein (Tata, 1975; Tata, 1976). When pure hevein (free of pseudo-hevein) was isolated and characterised, it was found to be a single peptide chain with glutamic acid at the N-terminus and a molecular weight of approximately 5,000 daltons (Tata, 1975; Tata, 1976). Later, an complete amino acid sequence of hevein was reported (Walujono *et al.*, 1976). It contained 43 amino acid residues in a single polypeptide chain and an estimated molecular weight of 4,729 daltons.

The microfibrilar protein: Dickenson (1963, 1965, 1969) in his ultrastructural studied and electron microscopic investigations of lutoids, first described some fibrillar components having a tightly coiled helical structure, which he named microfibrils. These structures were observed within lutoid of young latex vessels but were absent from mature vessels. These microfibrils

were later shown to be proteins containing upto 4% carbohydrate, and having an isoelectric pH about 4 (Audley, 1965; 1966). At ambient temperature (20°C), the microfibrils break up into smaller segments which reassemble on freezing (Audley, 1965; 1966).

The microhelices: These structures were first observed by Dickenson (1963) in lutoids from mature trees. However, Dickenson described them as stretched microfibrils. Later, Southorn and Yip (1968) and Gomez and Yip (1974; 1975; 1976) carried out detailed investigations and reported that these zig-zag structure differed from microfibrils in that they were larger in dimensions and were open helcies (not tigthly coiled helices of the microfibrils). They were called “Microhelices” by Gomez and Yip (1975). Lowering of the ionic concentration of B-serum by dialysis against water or by dilution of water resulted in the formation of micohelices (Tata, 1975; Gomez and Yip, 1974; 1975; 1976). Furthermore, their formation required the combination of two glycoproteins in a certain ratio. These are an acidic “assembly factor” (molecular weight 160,000) and a slightly basic “pro-helical protein” (molecular weight 22,000) (Tata, 1975). A third glycoprotein termed the “building factor” (molecular weight 5,000) appeared to promote the combination of single microhelices into bundles (Tata, 1980b). The “pro-helical protein” has some floculating activity on suspensions of rubber particles *in vitro.* Microhelices are rarely seen from young trees.

The basic proteins: The presence of basic proteins in B-serum was first demonstrated when B-serum or an aqueous extract of freeze-dried bottom fraction was electrophoresed (Tata and Edwin, 1970; Moir and Tata, 1960;

Karunakaran *et al.*, 1961). Two basic proteins – a major and a minor basic protein – which account for about 4% of the total proteins in latex were found to have lysozyme and chitinase activities (Tata *et al.*, 1983). The major basic protein has been crystallised and its molecular weight (approx. 26,000) determined. Its first 21 amino acid residues were elucidated, and found to differ significantly from those of hen egg, duck egg, baboon milk and T4 phage lysozymes (Tata *et al.*, 1983). The major basic protein, also referred to earlier as "band (I) first peak protein" (Fig. 6) was found to be identical with hevemine A, a cationic protein described by Archer (1976), another basic protein in B-serum.

Proteins in the lutoid membrane

Several proteins are present in the lutoid membrane, and many of these are active enzymes. One well-characterized membrane enzyme is ATPase (Moreau *et al.,* 1975). The electron transport activity of lutoid has been linked to ATPase, which is activated by several anions, this in turn leading to an accumulation of anions within the lutoid compartment. ATPase also operates as a proton pump to maintain proton gradients between the lutoid and latex cytosol- a function which was demonstrated by Chrestin and Gidrol (1986). Other membrane enzymes included NADH-cytochrome C reductase, this functions in an outward proton-pumping redox system that tends to reduce the concentration of protons in lutoid and hence acidify the cytosol (Moreau *et al.*, 1975). NADH-quinone reductase (d'Auzac *et al*., 1986) has also been described as being responsible for the production of superoxide ions.

Recently, the enzyme HMG-CoA reductase has been purified from lutoid membrane by solubilization with mild detergent (Wititsuwannakul *et al.* 1990). Characterization of the purified enzyme was carried out by determining its molecular structure and properties. The native enzyme was found to be a tetramer of four 44 kDa subunits-as found for other plant speciments-and membrane bound (Bach, 1986).

Lipids and phospholipids

Lipids and phospholipids associated with the rubber and non-rubber particles in latex play a vital role in the stability and colloidal behaviour of latex. Earlier studies (Cockbain and Philpott, 1963; Blackly, 1966) demonstrated that the rubber particles are strongly protected by a complex film protein and lipid material. It is believed that some of the lipids are present within the rubber particle. The concentration and distribution of lipids between the rubber cream and the bottom fraction had been studied (Ho *et al.*, 1976). These lipids were isolated and divided into neutral lipids and phospholipids for further analysis. There appeared to be distinct variation in the amount of neutral lipids extractable from rubber cream and from bottom fraction. Colloidal stability of latex was found related to the natural lipid content of rubber particles (Sherief and Sethuraj, 1978). Lutoids from different clones, however, were qualitatively similar. Triglycerides and sterols were the main components of the neutral lipids of rubber particles, whilst sterols and long-chain free fatty acids mainly made up the neutral lipids of the bottom fraction. A furanoid fatty acid containing a methylfuran group was found mainly in the triglyceride fraction of the neutral lipids (Hasma and Subramaniam, 1978). It constituted about 90% of the total

esterified acids. It was suggested that the main triglyceride in *Hevea* latex contained three furanoid fatty acids, hence making it a rare triglyceride known in nature. The phospholipid content of the rubber particles (approx. 1% on the weight of rubber) was similar between different clones. The total phospholipid content of bottom fraction was much less (only about 10%) than that in the rubber cream. It was suggested that the amount of neutral lipid (especially triglycerides) associated with the rubber particles was inversely related to the plugging index of the clone which the latex originated from (Ho *et al.*,1976). Lutoid stability, as indicated by bursting index, was found to be negatively correlated with the phospholipid content of the bottom fraction of latex (Sherief and Sethuraj, 1978).

A systematic study of the glycolipids from natural rubber was reported (RRIM, 1980). The glycolipids fraction was found to consist mainly of esterified sterol glucoside (ESG), monogalactosyldiglyceride (MGDG), sterol glucoside (SG) and digalactosyldiglycerate (DGDG). The sterol attached to ESG and SG was mainly β-sitosterol, while the acid components of ESG, MGDG and DGDG were of 14:0; 16:1; 18:0; 18:1; 18:2; 18:3 and furanoic acids. The constituents of the phospholipids are mainly phophatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl inositol (PI).

1.3 Latex Concentrate

1.3.1 The production of NR latex concentrate

On tapping the *Hevea brasiliensis* trees, natural rubber latex exudes, which has a rubber content between 25-40% by weight. The variation is due to factors such as the clone of the tree, the tapping system, the soil condition

and the season. The latex is normally called “field latex” and its average rubber content is 30% by weight. This material is not utilized in its original form due to its water content and susceptibility to bacterial attack. It is necessary both to preserve and concentrate it, so that the end product is stable and contains 60% or more of rubber.

Preservation of latex

For effective preservation of latex against precoagulation, factors affecting the stability of latex are to be understood and taken care of. There are several theories postulated for the auto-coagulation of latex, out of which the following assume importance.

Natural rubber latex contains about 4 to 5% non-rubber constituents consisting mainly of carbohydrates, proteins and lipids. Bacterial proliferation occurs at the expense of these non-rubber substances, resulting in the formation of acids, particularly volatile fatty acids (VFA). As VFA content increases coagulation of latex occurs (John, 1974). A second theory postulates that hydrolysis of various lipids present in latex liberates higher fatty acid anions which get adsorbed onto the surface of the rubber particles, possibly by replacing the adsorbed proteins. The free fatty acid anions interact with metallic ions such as calcium and magnesium, which are either present in latex initially or are gradually released by the action of enzymes. The formation of insoluble higher fatty acid soaps of calcium/magnesium draws the rubber particles together resulting in coagulation (Madge *et al.*, 1950). Another theory suggests that a proteolytic enzyme, coagulases, hydrolyses the protective layer of proteins

surrounding rubber particles, thereby exposing the surface of the particles which leads to coagulation (Woo, 1973).

When it is required to preserve latex for a few hours, chemicals such as formalin, sodium sulphate and ammonia at very low dosages of 0.02, 0.05 and 0.01%, respectively on latex are employed. These are termed anti-coagulants and are used to prevent precoagulation in the field (Cook, 1960).

Since 1853, ammonia has been recognised as the most effective and popular preservative for NR latex as it fulfills most of the requirements for an ideal preservative. However, this chemical has certain drawbacks as well. To be effective for longer periods, a higher dosage is to be used. Higher levels of ammonia in latex also leads to atmospheric pollution. Because of this problems, preservation systems comprising of low levels of ammonia, in combination with other chemicals were introduced (Cheong and Ong, 1974; John *et al.*, 1986). The commercially available low ammonia systems are those containing sodium pentachlorophenate (SPP), zinc diethyldithiocarbamate (ZDC) or boric acid along with 0.2% by weight of ammonia. The main drawback of these systems are high toxicity, lower mechanical and storage stability, poor chemical stability and slow rate of cure respectively for the SPP, ZDC and boric acid preserved latex. A composite preservation system consisting of tetramethlthiuram disulphide, zinc oxide and ammonia, popular known as the LA-TZ system was introduced during 1975 (John *et al.*, 1976) and subsequently commercialised. But this system has the drawback that the chemicals TMTD and zinc oxide are to be prepared as dispersions and in many cases they sediment during long term storage.

Concentration of latex

Three methods of concentration are employed, centrifugation, evaporation and creaming; centrifugation is the preferred method and accounts for 95% of total production.

Different types of centrifuges have been commercially available. The basic design of these machines is similar and it consists of a rotating bowl in which a set of concentric conical metallic separator discs are enclosed (Fig. 7 and 13). Latex enters the bowl through a central feed tube and passes to the bottom of the bowl through a distributor. A series of small holes on the separator discs, positioned at definite distance from the centre, allow the latex to get distributed and broken up into a number of thin conical shells within the bowl which rotates at high speed. At steady state running of the machine, the DRC of the latex at the periphery of the bowl will be much lower than that of the latex at the centre. The latex concentrate which has above 60% DRC flows towards the axis of rotation and is collected through a galley at the top. The skim latex which contains about 6 to 10% rubber is collected through a separate galley. The DRC of the latex concentrate and that of the skim depends on several factors such as speed of rotation of the bowl, pressure head in the feed cup, diameter of feed tube, length and diameter of the skim screw and DRC of the feed latex. Shorter skim screw, low feed rate of latex and higher DRC of field latex flavours a higher DRC for the concentrate. Factors affecting efficiency of different types of machines have been studied in detail and operating conditions to get the maximum bowl efficiency have been worked out (Piddlesden, 1940).

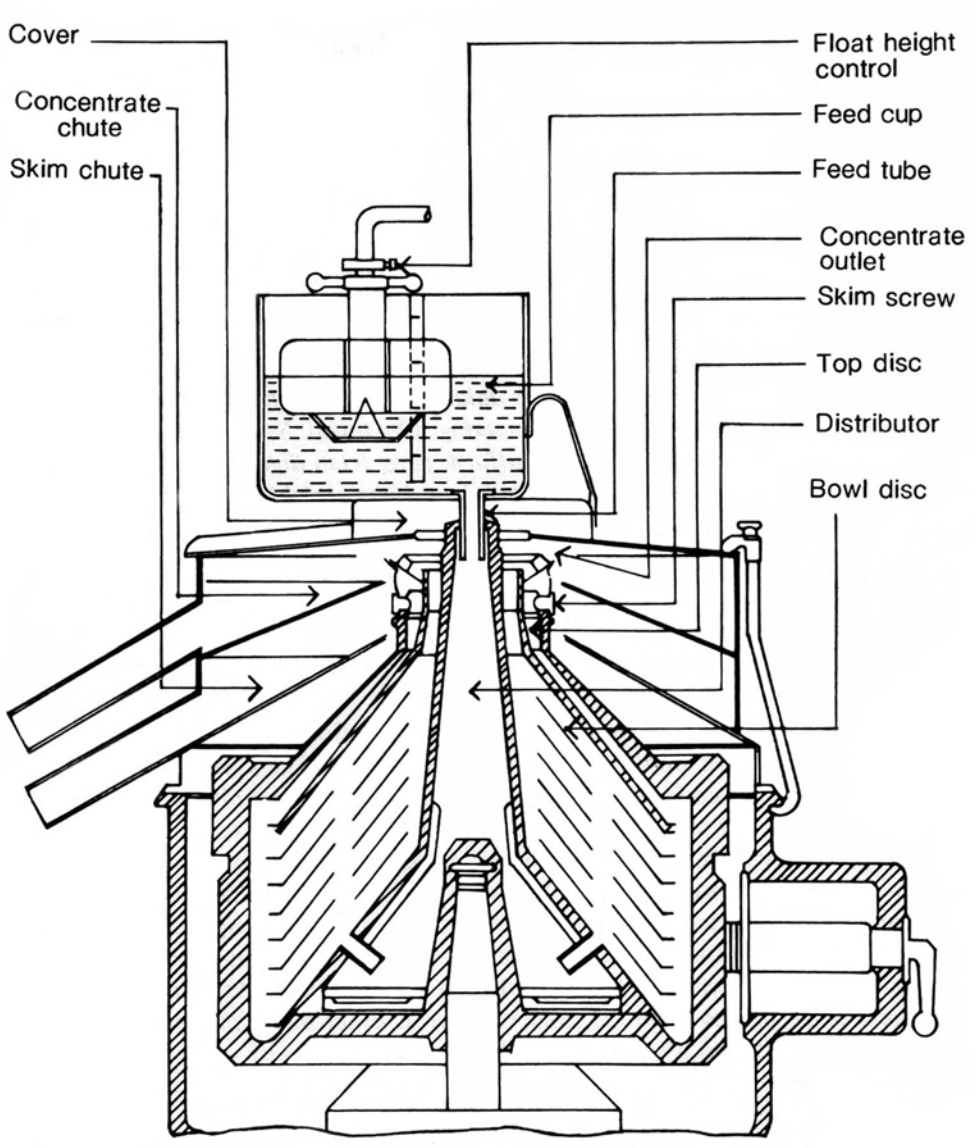

Fig. 7 A typical Alfa-Laval centrifuge bowl assembly.

Latex for centrifuging is to be adequately preserved against bacterial growth and development of volatile fatty acids. The dosage of ammonia required for preserving the field latex depends on the period of storage before centrifuging operation and varies from 0.3% to 1.0%. The preserved latex is treated with calculated quantity of diammonium hydrogen phosphate to remove magnesium ions as magnesium ammonium phosphate. On storage, this material settles at the bottom of the tank as sludge. Desludged latex is fed into the centrifuging machine at a constant rate.

Since most of the preservatives used in the feed latex would be lost in the aqueous phase of skim, it is necessary to supplement the concentrate with the required quantity of preservative depending upon the type of preservation system adopted. The concentrate may be preserved with ammonia alone or with a combination of ammonia with chemicals such as boric acid, sodium pentachlorophenate, zinc diethyl dithiocarbamate, zinc oxide, tetramethlthiuram disulphide etc. A small quantity of a higher fatty acid soap such as ammonium laurate is also normally used to boost the mechanical stability of the latex. Each of the above mentioned preservative systems has been studied in detail and the conditions for effective preservation of the concentrate standardised (Bloomfield and Mumford, 1960; Angove and Pillai, 1965; Poh, 1983; John *et al.*, 1986). Details of the preservation system used in centrfuged concentrate are giving in Table 1, the predominant latices are the HA and LA-TZ type (Gazeley *et al.*, 1988).

Table 1. Types of preservative system used in centrifuged NR latex concentrate (Gazeley *et al.*, 1988).

Designation	Abbreviation	Market share (%)	Preservative system (% by weight)
High or full ammonia	HA	66	0.7% ammonia
Low ammonia TZ	LA-TZ	17	0.2% ammonia, 0.025% zinc oxide, 0.025% tetramethylthiuram disulphide
Low ammonia pentachlorphenate	LA-SPP	6	0.2% ammonia, 0.2% sodium pentachlorphenate
Low ammonia boric acid	LA-BA	5	0.2% ammonia, 0.24% boric acid

1.3.2 Composition

Non-rubber materials in the serum

The non-rubber materials in the serum from latex concentrates comprise a wide variety of chemical species. The major constituents are known to be proteins and their composition products, fatty acid soaps and a range of organic and inorganic salts.

On processing the fresh latex to HA latex concentrate, Hasma (1994) indicated that a fewer number of proteins is present in the HA latex serum compared to a great variety of them in fresh latex serum. This is largely due to the loss of the proteins in skim latex and sludge and to some denaturation or degradation by ammonia. The HA latex serum proteins remaining are mainly anionic with molecular weight less than 14.0 kDa.

The amounts of fatty acid soap in the serum are relatively small since these surface active materials will be concentrated at the particle interface. The salts in latex concentrate serum have been analysed in some detail (Gorton and Pendle, 1986b; Archer *et al.*, 1963b) and 14 anions have been detected (Table 2).

The predominant cation present in natural latex is ammonium, NH_4^+, although the serum also contains significant amount of potassium (Gordon and Pendle, 1986b; Archer *et al.*, 1963b). On average potassium appears to represent about 25-35% of the total cation present. Low concentrations of sodium are also found in latex together with trace of rubidium.

The overall compositions of both HA and LA-TZ latices are summarised in Table 3, for both the latex and the total solids film.

Table 2. Average anion concentration found in mature latex concentrate sera (Gordon and Pendle, 1986b; Archer *et al.,* 1963b).

	Latex type	
Anion	HA	LA-TZ
Carbonate	22.25	22.0
Acetate	2.81	8.74
Malate	9.28	10.14
Succinate	5.45	2.03
Citrate	5.04	6.94
Formate	4.67	3.13
α-glycerophosphate	2.81	4.24
Glucose-1-phosphate	2.24	0.31
Phosphate	2.01	2.22
Oxalate	1.16	1.22
Chloride	1.10	2.01
Sulphate	0.58	0.63
Hydroxide	0.51	0.14
Propionate	-	5.34

Table 3. Overall composition in percentage by weight of latices and total solids films (Gazeley *et al.*, 1988).

	HA		LA-TZ	
	Latex	TSC	Latex	TSC
Rubber[1]	59.67	97.61	59.61	97.62
Proteins, etc[2].	1.06	1.73	1.03	1.69
Soaps[3]	0.23	0.38	0.23	0.38
Salts	0.40	0.28	0.38	0.32
Ammonia	0.68	-	0.21	-
Water	37.96	-	38.54	-

[1] As measured by DRC test.
[2] Includes carbohydrates, amino acids, sugars.
[3] Calculated as ammonium stearate.

Adsorbed non-rubber materials

When in the tree, the particles in natural latex are presumed to possess an adsorbed layer of a protein-lipid complex, similar to the membrane of many biological cells (Gorton and Pendle, 1986a). As a consequence of the addition of ammonia to preserve the latex concentrate, the lipid materials are believed to hydrolyse slowly, releasing fatty acid soaps which themselves become adsorbed onto the particle surface. The adsorption of these soaps is thought to account for the spontaneous rise in mechanical stability when ammoniated concentrate is stored (Chen and Ng, 1984).

The hydrolysis of lipids is often assumed to be complete in mature concentrate, but some indirect evidence suggests that neutral lipids may still be present (Chen and Ng, 1984). Also, a high proportion of the phosphorus detected in latex can not be identified in the serum and may possibly be present on the particles as phospholipids (Gorton and Pendle, 1986a; Archer *et al.*, 1963b).

The fatty acids in latex have been analysed (Chin *et al.*, 1978; Jurado and Mayhan, 1985) and as many as nine have been identified. A new fatty acid, 10,13-epoxy-11-methyloctadeca 10, 12-dienoic acid, has been found both in fresh latex and mature concentrate (Jurado and Mayhan, 1985; Hasma and Subramaniam, 1978; Chen and Ng, 1984).

2. Natural Rubber Latex Allergy

2.1 Definition and Symptoms

Latex allergy is an immunoglobulin IgE mediated hypersensitivity to protein present in natural rubber latex with contact urticaria to latex gloves as paradigm. In contrast, delayed hypersensitivity to rubber latex is cause by accelerators or antioxidants added to cure natural latex or synthetic rubber with allergic contact dermatitis to rubber gloves as the paradigm (Slater, 1992; Taylor, 1993).

The typical allergic reaction to latex protein is characterized by pruritis, erythema and edema, as is common with other allergen-IgE, mast cell sensitivities (Forstrom, 1980; Granady and Slater, 1995; Ownby, 1995). As sentivity and exposure to allergen increases, urticaria may also develop, initially restricted to the site of the latex contact, but it may eventually spread to the contiguous area of the skin and finally become systemic (Frostrom, 1980; Meding and Fregert, 1984; Pecquet *et al.*, 1990; Ownby, 1995). Airborne exposure may lead to nasal, ocular and pulmanary symptoms (Sondheiner *et al.*, 1989; Turjanmaa and Reunala, 1989; Lagier *et al.*, 1990; Baur *et al.*, 1993; Tomazic *et al.*, 1994; Kujala *et al.*, 1996). The ocular symptoms usually start with pruritis and progress to tearing, chemosis and edema (Sussman *et al.,* 1991; Swanson *et al.*, 1994; Granady and Slater, 1995; Ownby, 1995). Frequently, direct contact with latex products leads to sudden swelling of the eyelids. Nasal symptoms include sneezing, watery rhinorrhea and congestion. Patients may develop sore throat, irritation of the larynx or cough (Lagier *et al.*, 1990; Kujala *et al.*, 1996). Pulmanary symptoms may range from coughing to life-threatening

asthma (Forstrom, 1980; Granady and Slater, 1995). Lung function changes and chest symptoms may or may not be present in patients exposed to airborne latex allergens (Tarlo *et al.*, 1990). Gastrointestinal, cardiovascular and genitourinary symptoms from latex allergy have been well documented (Morales *et al.*, 1989; Nguyen *et al.*, 1991; Sussman *et al.*, 1991; Ownby, 1995). The severe forms of latex allergy are characterized by wheezing, stridor, sneezing rhinorrhea, ocular itching, urticaria, hypertension and anaphylaxis (Ownby, 1995). Although not very common, latex-induced anaphylaxis occurs soon after exposure to the allergens and even with prompt intervention latex-induced anaphylaxis can be fatal (Gerber *et al.*, 1989; Leynadier *et al.*, 1989; Schwartz and Zurowski, 1993).

2.2 Epidemiology and Risk Factors

At present, the incidence of NRL allergy is still unknown, but several prevalence studies have been published. In European health-care workers screened with skin prick testing (SPT), the prevalence of NRL allergy has range from 2.8% to 10.7% (Turjanmaa, 1987; Arellano *et al.*, 1992; Yassin *et al.*, 1994; Turjanmaa *et al.*, 1995a). In agreement with SPT screening, a serologic study base on RAST found a 5.5% prevalence of NRL allergy among 381 hospital workers in the USA (Kaczmarek *et al.*, 1996). Children with spina bifida have shown the highest prevalence of NRL allergy. The frequency has ranged from 32% to 51% in SPT screenings (Kelly *et al.*, 1993; Moneret-Vautrin *et al.*, 1993; Slater, 1994). In contrast to these studies, a low prevalence rate of 4.3% was reported for Venezuenlain children with spina bifida (Capriles-Hulet *et al.*, 1995). The occurrence of NRL allergy has not been systematically surveyed among the general population, but the prevalence seems to be clearly

less than 1% (Arellano *et al.*, 1992; Moneret-Vautrin *et al.*, 1993; Hadjiliadis *et al*., 1995; Turjanmaa *et al.*, 1995b).

In addition to repeated exposure to gloves and other NRL products, atopy seems to be the principle determinant for the development of NRL sentisization. In agreement with this, NRL-allergic health care workers are atopic 2.2-4.2 times more often than their coworkers without NRL allergy (Turjanmaa, 1987; Arellano *et al.*, 1992; Moneret-Vautrin *et al.* 1993; Yassin *et al.*, 1994). Hand eczema disrupts the barrier and, together with personal atopy, this condition is one of the main predisposing factors of NRL allergy (Wrangsjo *et al.*, 1988; Jaeger *et al.*, 1992; Charous *et al.*, 1994; Taylor and Praditsuwan, 1996). In addition to contact with gloves, the patients may also react to airborne NRL in their working environment (Tarlo *et al.*, 1990).

Children with spina bifida form a well-known, prominent risk group for NRL allergy (Slater, 1989; Moneret-Vautrin *et al.* 1993; Swanson *et al.*, 1994). Many of these NRL-allergic children have had associated food allergy to cereals, banana and other fruits (Yhitalo *et al.*, 1996). These findings suggest that a pre-existing food allergy could be an additional risk factor for NRL allergy in children.

2.3 Diagnosis of NRL Allergy

2.3.1 Skin Tests

In Europe, the open patch test and scratch-chamber test were the first methods used in the diagnosis of NRL allergy. Later, prick testing became more popular, and in addition to glove pieces and eluates, ammoniated and non-ammoniated NRL were used as skin prick test material (Turjanmaa, 1988). In

one report, nonstandardized allergic preparations were compared in prick testing, and a good correlation was found between the glove eluates, non-ammoniated latex and crushed rubber-tree leaves (Turjanmaa *et al.*, 1988b).

Wrangsjo *et al.* (1988) investigating 30 patients with contact urticaria, obtained positive skin tests with a solution of rubber latex that had been extracted in saline (1:9 w/v), centrifuged, sterized by filtration and diluted 1:100 w/v in saline containing 0.03% human serum albumin.

Turjanmaa *et al.* (1988a) performed skin prick tests in 40 latex allergic subjects with extracts of 19 brands of latex surgical and cleaning gloves. Twenty-six of the subjects had been sentisized by surgical gloves and the remaining 14 by cleaning gloves. The results confirm that allergic proteins persist in various sorts of latex glove after being manufactured from natural rubber latex and that these allergens can cause contact urticaria. However, the frequency of positive test reactions varied from 8 to 100%, depending on the source of the extract, indicating that there are differences in the quality or the amount of latex allergens eluting from different brands of gloves.

There are several commercially available nonstandardized allergens in Europe and two of these NRL allergens have been compared with an allergenic glove eluate (Triflex, Baxter). A group of 110 patients with NRL allergy was tested; the sensitivities of the commercial allergens were 88% (Staller-gènes) and 54% (ALK), and that of a reference glove eluate was 92% (Turjanmaa *et al.*, 1994). The specificity of all test materials was 100% because none of the 200 control patients not allergic to NRL had positive reactions.

2.3.2 Use or Challenge Tests

In Finland and Sweden (Turjanmaa *et al.*, 1995b), the use test with latex gloves is performed when there is a discrepancy between SPT results and clinical history or when in vitro studies give positive reactions and the patient has not noticed any symptoms after using rubber products.

Turjanmaa *et al.* (1988b) reported that a use test in which a latex glove was worn on a damperred hand for 15 minutes gave positive results in 12/13 cases; the thirteenth patient, who had not worn latex gloves during the previous 4 years, had a positive response only after wearing the glove for 4 hours. None of these allergic patients reacted to contact with a vinyl glove, and control employees without contact urticaria did not react to the latex glove.

Wrangsjo *et al.* (1988) tested 10 patients with a history of systemic symptoms by placing 1 cm^2 pieces of surgical glove latex material moistened with saline on the skin of the forearm, or by having them wear one glove finger, for 20 minutes. A positive reaction was observed in 9/10 patients, two of whom experienced only distant symptoms (conjunctivitis, rhinitis, dyspnea, facial redness) during the test.

Jaeger *et al.* (1992) tested seven patients allergic to latex gloves with six different brands of sterile gloves, including three “hypoallergic” brands and two latex-free brands. The latex-free gloves were tolerated, but the “hypoallergic” gloves were not.

These authors also point out that it is difficult to standardize provocation tests because of different reports from patients and the unknown allergen content in rubber materials, as well as differences in the penetration of

allergens into the skin (Turjanmaa *et al.*, 1988b; Wrangsjo *et al.*, 1988; Jaeger *et al.*, 1992).

Inhalation provocation tests have been done with latex to confirm the presence of respiratory symptoms induced by contact with latex or airborne latex particles. Jaeger *et al.* (1992) performed such tests with 18 patients who had experienced dyspnea or rhinitis/conjunctivitis related to latex contact. Inhalation of cornstarch from latex gloves in a small inhalation chamber during 5-60 minutes induced reactions in all 18 patients tested. The most frequent reactions during the test were rhinitis, cough and conjunctivitis. A significant increase in airway resistance was seen five cases. One patient developed severe facial angioedema and hypotensis; another complained of generalized malaise. These symptoms were not induced by handling of constarch-powdered, latex-free gloves.

2.3.3 Blood or *in vitro* Tests

A latex radioallergosorbent test (RAST) is available commercially for the *in vitro* detection of latex IgE antibodies (Latex, k82, Phamacia Diagnostic), but, it is less sensitive than skin tests and provocation challenge, detecting these antibodies in only about 50-70% of skin test-positive, latex allergic patients (Frosch *et al.*, 1986; Axelsson *et al.*, 1987; Turjanmaa *et al.*, 1988b; Wrangsjo *et al.*, 1988; Jaeger *et al.*, 1992).

Jaeger *et al.* (1992) found that in 16% of their allergic patients, specific IgE antibodies were detected by RAST only when the test was made with latex-coupled disks prepared in their laboratory.

Sandberk *et al.* (1992) reported that 39% of a group of 31 in a spina bifida clinic had a positive RAST. Three of these children had had an allergic reaction to latex, whereas no child with a negative test had a clinical history of hypersensitivity.

A new latex-specific fluorescent enzyme immunoassay for the detection of latex-specific IgE (Phamacia CAP System, PCS) was evaluated by Dolen *et al.* (1992). Mathew *et al.* (1992) obtained a positive result with this assay in 4/10 skin test positive, latex sensitive spina bifida patients.

Kwittken *et al.* (1992) have developed a flow cytometric assay (FCA) for antilatex IgE antibodies and report an excellent correlation between FCA and RAST and between FCA and ELISA values.

A patients' IgE response to NRL antigens has also been evaluated with immunoblotting (Alenius *et al.*, 1991; Alenius *et al.*, 1993; Alenius *et al.*, 1994a; Alenius *et al.*, 1994c) and immunoelectrophoretic methods such as cross radioimmunoelectrophoresis (Makinen-Kiljunen *et al.*, 1992). A sensitivity of 81% has been demonstrated with the immunoblot method (Alenius *et al.*, 1994c). In addition, the histamine-release test has been found to be sensitive in the evaluation of latex-specific IgE antibodies in vitro (Carrillo *et al.*, 1986; Turjanmaa *et al.*, 1989). In one study, 13 of 14 (93%) samples were found to be positive (Turjanmaa *et al.*, 1989) and another study reported positive results in 11 of 16 (69%) cases (Leynadier and Dry, 1991).

2.4 Latex Allergens

2.4.1 Rubber Elongation Factor (Hev b 1)

Hev b 1 was the first latex allergen that was characterized on the molecular level. Czuppon *et al.* (1993) isolated a 58 kDa protein from raw latex and latex gloves. The allergen was a monovalent homotetramer molecule, in which the 14.6 kDa monomer was identified by ammino acid composition and sequence homologies of tryptic peptides as the rubber elongation factor found in natural latex of the Malaysian rubber tree. Hev b 1 is a highly hydrophobic protein tightly bound to large rubber particle (diameter>350 nm) purified from *H. brasiliensis* latex and is necessary for prenyltransferases from a number of sources to add multiple cis-isoprene units to rubber molecules. Hev b 1 account for 10-60% of the total protein in whole latex but is absent in C-serum, the supernatant fluid obtained when rubber particles are removed by centrifugation (Dennis and Light, 1989). The amino acid sequence of Hev b 1 was determined and deposited in the database by Dennis *et al.* (1989).

Alenius *et al.* (1996b) reported a relatively low frequency of IgE antibodies to purified Hev b 1 with the exception of spina bifida patients. In ELISA 4/39 (10.3%) latex allergic patients had IgE antibodies to purified Hev b 1 and sera from 4/6 (67%) latex allergic children with spina bifida or other congenital abnormalities exhibited IgE antibodies to Hev b 1 (Alenius *et al.*, 1996b). Yeang *et al.* (1996) produced and tested the recombinant Hev b 1. The authors concluded that it was not a major latex allergen, because its ability to bind IgE in immunoblots was confined to two serum pools from spina bifida patients and not observed in 19 adults with clinical symptoms of latex allergy.

In contrast, Hev b 1 purified from latex sap induced lymphocyte proliferation responses in 12/23 (52%) latex sensitized hospital employees indicating that Hev b 1 is a relevant allergen for health care workers (HCWs) (Raulf-Heimsoth *et al.,* 1996). Sera from 11 latex allergic subjects including 3 from SB patients, 2 monoclonal antibodies raised against purified Hev b 1, and 3 rabbit antisera were employed to defined B-cell epitopes on Hev b 1 (Chen *et al.*, 1996b). The C-terminal portion of Hev b 1 and the region with amino acid residues 31-64 were determined as the most allergenic/antigenic. In both regions, the minimal IgE-binding epitope was almost identical to the IgG-binding epitope (Chen *et al.*, 1996b). Chen *et al.* (1997a) suggested that Hev b 1 was not only a major allergen for SB patients, but also an important latex allergen for HCWs. 50/62 (81%) SB patients with IgE to latex allergens had Hev b 1 specific IgE, and 52/103 (50%) latex allergic HCWs had IgE that bound to Hev b 1.

2.4.2 β-1,3-Glucanase (Hev b 2)

A group of proteins called pathogenesis-related (PR) proteins are synthesized by plants in response to microbial infection. Several PR proteins have been identified as β-1,3-glucanase. Sequences of several distinct tobacco β-1,3-glucanase isoform, both acidic (extracellular) and basic (intracellular), have been reported and the regulation of the gnl isoform has been studied (Castresana *et al.*, 1990). Clones encloding a basic β-1,3-glucanase were isolated from *H. brasiliensis* using the *Nicotiana plumbaginifolia* cDNA clone gnl as a probe. Nucleotide sequence analysis showed that a 1.2 kb *Hevea* cDNA encoding a basic β-1,3-glucanase had 68% nucleotide similarity the gnl cDNA (Chye and

Cheung, 1995). The presence of an N-terminal extension (residues 1-36) and a C-terminal extension (residues 353-374) suggested it belongs to class-I β-1,3-glucanase. Genomic Southern analysis indicated the presence of a low copy gene family encoding β-1,3-glucanases in *Hevea brasiliensis.*

Alenius *et al.* (1995a) purified a 36 kDa protein to homogeneity by HPLC gel filtration and reversed phase chromatography. The sequences of these trypic peptides showed similarities from 67 to 83% to several plants endo β-1,3-glucanases. Sequences of the peptides SWVQK and YIAVGNEISPVNR were identical to portions of the sequence published by Chye and Cheung (1995), whereas the peptide SYLNPIIR differed in two amino acid residues. The purified 36 kDa protein bound IgE from 6/29 (21%) adult latex allergic patients'sera, but did not react with IgE from 6 SB patients (Alenius *et al.*, 1995a).

The basic β-1,3-glucanase isolated from the B-serum of NRL was first described as allergen Hev b 2 by Sunderasan *et al.* (1995). Hev b 2 appeared as a doublet of 34 kDa in SDS gels and both bands were recognized by a monoclonal antibody. IgE from one latex-allergic patient was shown to bind Hev b 2. Isoelectric focusing of Hev b 2 revealed a single spot at pI 9.5. The N-terminus of Hev b 2 was blocked, but cyanogen bromide digests produced peptides which were sequenced. One 11 residue peptide FDENNXQPEVE was 90% identical to published sequence and an other 13 residue peptide PNIHDAIRSAGLQ was identical (Sunderasan *et al.*, 1995). Both peptide sequence also showed a good similarity to the published sequence of endo-β-1,3-glucanases from *N. plumbaginifolia*, *N. tabacum* and tomato.

2.4.3 Hev b 3

Alenius *et al.* (1993) examined sera from 15 SB children and 5 children with other congenital anomalies for IgE antibodies to NRL antigens. IgE antibodies in 10/12 (83%) US SB patients and in 2/3 Finnish SB patients recognized a previously undescribed 27 kDa allergen. Two patients with other congenital anomalies also had IgE to the 27 kDa allergen. IgE antibodies to the 27 kDa allergen were not detected in other latex allergic patients. Alenius *et al.* (1995b) purified the 27 kDa protein from NRL by HPLC. As the N-terminus was blocked the protein was digested with trypsin and the purified tryptic peptides were subjected to sequence analysis. Six of the 14 sequenced peptides (number 3,8,11a and b,13 and 14) showed sequence similarities to stretches within the first 130 amino acid residues of Hev b 1.

Similarly, Lu *et al.* (1995) reported a polypeptide (MW 23 kDa; pI 4.8) to be closely associated with hypersensitivity in SB patients. The 90 amino acids sequenced from this 23 kDa showed 45% similarity with Hev b 1, and it shared identical amino acid sequence motif with the 27 kDa protein described by Alenius *et al.* (1995b). As the data profiles of both proteins match each other well it can be assumed that both research groups investigated the same protein. The purified 23 kDa polypeptide reacted with IgE from 13/17 (76%) latex allergic SB patients, but from only 1/5 HCWs with latex allergy. Furthermore, all the sera of patients with anaphylaxis to latex showed reactivity with the 23 kDa polypeptide.

Yeang *et al.* (1996) described a 24 kDa protein found mainly on rubber particles (average diameter 70 nm), which was recognized by IgE from

latex allergic SB patients and named it Hev b 3. Hev b 3 has a tendency to fragment into several polypeptides of lower molecular weight (from 24 to about 5 kDa) even when stored at -20°C. Hev b 3 fragments, resulting either from storage or by reacting with B-serum, are electrophoretically discrete bands indicating that fragmentation occurs at predeterminated sites on the molecule.

2.4.4 a Microhelix Component (Hev b 4)

Hev b 4 was isolated from NRL B-serum (Sunderasan *et al.*, 1995). This acidic protein has a pI of 4.5 and appeared under reducing condition as a broad band of about 50-57 kDa. One latex allergic patient's serum was used in an immunoblot to show IgE binding to this band. Immuno-gold labelling with the monoclonal antibody USM/RB 3 showed that Hev b 4 was a component of the microhelix protein complex of B-serum. Amino acid sequencing of Hev b 4 revealed an N-terminal of ELDEYLFS- FGDGLYDAGNA, which did not match any previously published amino acid sequences. The significance of Hev b 4 as an allergen has not yet been determined.

2.4.5 Acidic Protein (Hev b 5)

Hev b 5 was found by Akasawa *et al.* (1996a) as an acidic protein in extracts prepared from latex gloves, which were shown to elicit allergic reactions. From a latex cDNA library, the cDNA coding for this protein was isolated and sequenced. The deduced amino acid sequence showed a high degree of homology to an acidic protein identified in kiwi fruit (*Actinidia deliciosa* var. *deliciosa*). The sequence homology (47% sequence identity) between these two acidic proteins suggested a possible molecular explanation for the the high frequency of allergic reactions to kiwi fruit in latex allergic patients. However,

the kiwi fruit protein appears in large quantities only during the early stages of fruit development.

Simultaneously, Slater *et al.* (1996) isolated a cDNA clone by utilizing IgE from the serum of a latex allergic HCW to screen a cDNA library from *Hevea* latex. The Hev b 5 cDNA encoded an reading frame of 152 amino acid residues. The nucleotide and deduced protein sequences had significant similarity to an acidic (pI 3.5) 18.9 kDa protein (SWISS-PROT P43393) from kivi which is known to cause allergic reactions in some latex allergic patients. Hev b 5 was expressed as the fusion protein rHev b 5/MBP. The uncleaved fution protein bound IgE in RAST in 12/13 (92%) latex allergic HCWs and in 32/57 (56%) of SB patients.

2.4.6 Prohevein (Hev b 6) and Hevein

A 20 kDa NRL protein, previously shown to most important for adult NRL allergic patients (Alenius *et al.*, 1993; 1994c), was identified by amino acid sequencing as prohevein (Alenius *et al.*, 1995a). In immunoblotting, purified prohevein bound IgE from 24/29 (83%) and in ELISA from 36/52 (69%) sera of NRL allergic patients (Alenius *et al.*, 1995a; 1996b). Purified prohevein also elicited positive SPT reactions in 6/7 NRL allergic patients and was considered to represent a major NRL allergen (Alenius *et al.*, 1995a). In line with these findings, Beezhold *et al.* (1994) showed that the amino acid sequences of two NRL protein bands excised at 14 and 18 kDa regions were homologous to prohevein. However, the authors did not assess the IgE reactivity of patient sera against purified proteins.

Attempts have also been made to localized IgE binding epitopes of prohevein. Alenius *et al.* (1996a) reported that around 70-80% of NRL-allergic patients had IgE antibodies to purified prohevein, whereas a minority (15-20%) of these patients had IgE against the purified prohevein C-domain in ELISA and immunoblot assay. Moreover, the IgE-binding peptides purified from a brand of highly allergenic NRL gloves were shown to be hevein moleculular since they showed amino acid sequences identical to those of the prohevein N-terminus and had a molecular mass corresponding to that of hevein (4719.1 Da), a 43-amino acid N-terminal fragment of prohevein (Fig. 8). In ELISA, 56% of 45 NRL-allergic patient sera showed IgE antibodies to purified hevein, and hevein elicited positive SPT reactions in patients showing IgE to the N-terminus of prohevein. The same study also showed that purified hevein inhibited 72% of IgE binding from pooled sera of NRL-allergic patients to solid-phase glove extract in ELISA inhibition. The authors concluded that the main allergenic epitope of prohevein is located in its N-terminus, and that the immunologically active fragments carrying this epitope, known as hevein, are found in highly allergenic latex gloves. A further study by anothor group (Chen *et al.*, 1996a) has confirmed hevein as a major NRL allergen.

2.4.7 Patatin-Like Protein (Hev b 7)

Beezhold *et al.* (1994) and Tomazic *et al.* (1995) detected an abundant 45 to 46 kDa protein that reacted very intensively with IgE antibodies of HCWs with established latex allergy. Beezhold *et al.* (1994) identified this protein as highly homologous to the patatin storage proteins encoded by a multigene family in potato and tomato and determined the N-terminal sequence

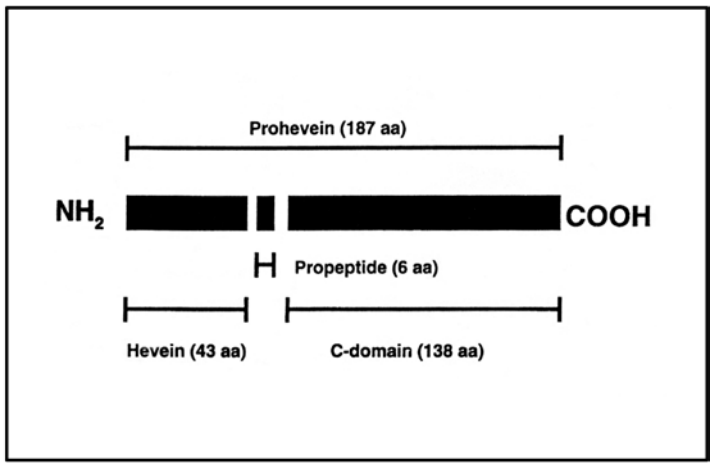

Fig. 8 Schematic composition of prohevein (hevein preproprotein), a major NRL allergen (Turjanmaa *et al.*, 1996a).

as LTQG-KKLTVFSID. 9/40 (23%) of latex allergic HCWs recognized this 46 kDa protein found in non-ammoniated latex.

In a further study, the intact 46 kDa protein and the 30 kDa cyanogen bromide fragment were analysed for primary structure (Beezhold *et al.*, 1996). The N-terminal amino acid sequence of the 46 kDa protein showed 60% sequence similarity to patatin, a protein from potato. Likewise, the 30 kDa cyanogen bromide fragment had 59% sequence similarity to an internal region from exon 2 of patatin. A cytosolic phospholipase A_2 from potato tissues, which is involved in signal transduction in the resistance reaction induced in potato by inoculation with the late blight fungus, appeared to be patatin (Senda *et al.*, 1996).

Hev b 7 was cloned and expressed in *Pichia pastoris* and not only shown to bind latex-allergic patients' IgE but also to possess esterase activity (Sowka *et al.*, 1998).

2.4.8 Latex Profilins (Hev b 8)

Profilin was frequently identified as an allergen in many plant species. Using IgE binding inhibition by purified profilins it was demonstrated that profilin is an IgE binding component in the cytosolic fraction of natural rubber, and to a lesser extent, in the rubber fraction (Vallier *et al.*, 1995). Thirty-five of 36 sera containing IgE to ragweed-profilin reacted with profilin from latex, indicating structural homologies between profilins from latex and ragweed. Because profilin is also present in banana extract, it is likely to involved in cross-sensitivity to banana and latex. In a group of 19 individuals allergic to latex only two had anti-profilin IgE antibodies. Profilin was barely detectable on glove extract immunoblots (Vallier *et al.*, 1995).

The present of cross-reactive IgE binding components in the different latex extract, extracts from birch pollen allergens (Bet v 2) was studied by immunoblot inhibitions and quantitative competition experiments (Fuchs *et al.*, 1997). The cross-reactive allergen profilin was purified from latex as an IgE binding component, but was only present in small amounts in latex extract. Nieto *et al.* (1998) purified profilins (10.2, 14.2 and 15.7 kDa) from latex and skin prick tested 24 SB patients. They found positive reactions in all patients. SPTs were negative in atopic patients with fruit allergy. A group of 17 adults allergic to latex had positive SPT reactions in 95% of the cases tested. At the

moment, the significance of latex profilin for the clinical manifestation of latex allergy is not clear.

2.4.9 Enolase, 2-Phosphoglycerate Dehydratase (Hev b 9)

Hev b 9 is an 51 kDa enzyme with very high homology with *Ricinus communis* and *Cladosporium* enolase (Posch *et al.*, *1997*; Achatz *et al.*, 1995). Posch *et al.* (1997) characterized several additional latex allergens by two-dimentional elctrophoresis and protein microsequencing. Protein spots '14c' and '15' (N-terminal sequence AITIVSVRARQIF) shared over 90% sequence identity with the N-terminal of *Ricinus communis* enolase (SWISS-PROT P42896). *Ricinus* belongs to the family Euphorbiaceae as does *Hevea*. The identity of the latex enolase sequence to the N-terminal sequence of a tomato enolase (SWISS-PROT P26300) was over 70%.

Interestingly, the allergen Cla h 6 (47 kDa, SWISS-PROT P42040) of the mould *Cladosporium herbarum* also is an enolase (Achatz *et al.*, 1995) . The cDNA of Cla h 6 encloned 441 amino acids. Cla h 6 shares 73% amino acid identity and 83% amino acid similarity with *Saccharomyces cerevisiae* enolase 1. Cla h 6 is a typical minor allergen of *C. herbarum* as it is recognized by IgE of 20% of all patients with a positive *Cladosporium* immunoblot. The *Ricinus* enolase shares 74.5% amino acid identity and 61% amino acid similarity with *Cladosporium* enzyme. Sequence similarities in this range may will be the cross for cross-reactivities between the *Cladosporium* and the *Ricinus* as well as the latex enolase.

2.4.10 Manganese Superoxide Dismutase (Hev b 10)

Manganese superoxide dismutase (MnSOD) has been detected in a number of fungi, bacteria and man (Miao and Gaynor, 1993; Crameri *et al.*, 1996). *Aspergillus fumigatus* MnSOD has been recognized as amajor allergen (Hemmann *et al.*, 1998). These allergens also showed strong PBMC stimulation (Crameri *et al.*, 1996). This antigen showed strong homology with *A. fumigatus*, *E. coli* and human MnSOD. The results from these few studies indicate that in spite of the homology with other MnSODs, this allergen showed only a low degree of cross-allergenicity with mold-allergic sera (Crameri *et al.*, 1996).

2.4.11 Hevamine

Hevamine is a 30 kDa basic protein from the lutoid of rubber latex and may play a role in plugging the latex vessels and cessation of latex flow. It is an enzyme with both lysozyme and chitinase activity and was isolated and purified from *H. brasiliensis* latex. The enzyme is homologous to certain pathogenesis-related (PR) proteins from other plants (Rozeboom *et al.*, 1990). The primary structure of hevamine was determined and the positions of three disulfide bridges were identified (Jekel *et al.*, 1991). The sequence had about 60% identity with that of a chitinase from cucumber and 95% with the N-terminal sequence of the lysozyme/chitinase of *Parthenocissus quinquefolia* (Jekel *et al.*, 1991). The three dimentional of hevamine was refined at 1.8 A° resolution and final model consists of all 273 amino acid residues and 206 ordered water molecule (Terwisscha van Scheltinga *et al.*, 1996). The purified hevamine bound IgE from only 1/29 latex allergic sera tested (Alenius *et al.*, 1995a).

2.4.12 Class-I Chitinase

Nearly all plant chitinases isolated to day are endochitinases, i.e. they hydrolytically degrade chitin from within the polymer rather than at its terminus (Graham and Sticklen, 1994). Class-I chitinases are basic and contain a cysteine-rich N-terminal domain with chitin-binding properties. The first indication of a 30 kDa cross-reactive allergen between latex, avocado and banana was given by Lavaud *et al.* (1995). Allergens of natural latex, latex gloves, avocado pear and banana extracts were investigated by immunoblotting sera from patients with latex and fruit allergies. In sera from patients with latex and fruit allergy, prominent IgE binding was revealed at about 30 kDa with latex and fruit extracts. Cross-inhibition of immunoblotting confirmed that this major allergen was linked to a common epitope present in latex and fruits.

Akasawa *et al.* (1996b) isolated and identified an avocado chitinase (approximately 30 kDa) that reacted with IgE antibodies from 14/22 (64%) patients allergic to latex. The full cDNA sequence of a major allergen of avocado, its IgE binding properties and its cross-reactivitiy with latex allergens were reported by Vanek-Krebitz *et al.* (1997). The cDNA clone obtained from a differential cDNA library coded for a class-I endochitinase. In immunoblotting, hevein-immunized rabbit serum identified a 33 kDa banana protein which also bound IgE-antibodies from 7/16 latex allergic patient sera (Mikkola *et al.*, 1997). In immunoblot inhibition, purified hevein completely inhibited IgE-binding to the 33 kDa banana protein. These results suggested that cross-reacting IgE-binding epitopes are shared by hevein and a 33 kDa banana protein.

2.4.13 Class-II Chitinases

Class-II chitinases are acidic, lack the N-terminal cysteine-rich domain and have a high sequence similarity to class-I chitinases within the catalytic domain (Graham and Sticklen, 1994). A 19 residues N-terminal amino acid sequence obtained by two dimensional immunoblot and microsequencing was published by Posch *et al.* (1997). A sequence alignment search for the peptide DIGSIISKSTFEEF-LKXGN obtained from 'spot 12' exhibited a high sequence homology to several plant chitinases.

2.4.14 Additional Latex Allergens

Lysozyme

Lysozyme were eluted from latex gloves or extracted from ammoniated latex and detected with a cell suspention-clearing test (Yagami *et al.*, 1995). A chromatographically separated lysozyme (27 kDa, pI 9.5) was investigated for its physicochemical and enzymatic properties and allergenicity. The purified lysozyme was enzymatically very similar to lysozyme of fruit such as fig, or papaya and was recognized by IgE from sera of 2 latex allergic individuals.

Papain

Baur *et al.* (1995) studied the cross-reactivity of IgE antibodies recognizing epitopes of latex allergens and papain. 8/24 latex-sensitized individuals had IgE to purified papain and 6/12 papain workers with IgE antibodies to papain revealed IgE to latex allergens. Each patient group had not previously been exposed to the allergens from the other source. These results indicate the present of common epitopes of papain and latex allergens.

Posch *et al.* (1997) characterized several additional latex allergens by two-dimensional electrophroresis and microsequencing. Peptides 16 (RKVDVDVX-VVPYT) and 17 (ARKFFVGG) demonstrated identities between 50 and 66% of spinach, rice and tomato triosephosphate isomerases. Peptide 17 showed high identity to the N-termini of several triosephosphate isomerase from plants including *Arabidosis thaliana* and rice. Sequence alignments of spot 19 (ANWSPYDNNGG) revealed similarities to several proteasome subunits.

2.5 Allergens in NRL products

Immunoblot assays have been used to analyze IgE-binding proteins in NRL products. In a study of eight different glove brands, a total of 14 protein bands, ranging from 11 to 200 kDa, could be identified (Alenius *et al.*, 1994b). Five brands showed positive immunoblot reactions when tested with IgE antibodies from sera of NRL-allergic patients. The strongest reactions were to 14 and 30 kDa allergens. However, the authors speculated that, because of the limitations of the immunoblot assay, it is possible that the glove extracts contained IgE-binding low molecular mass (<10 kDa) peptides that may have escaped detection. It is possible that new allergenic epitopes are formed during glove manufacturing. Evidence of this was provided by Makinen- Kiljunen *et al.* (1992), who demonstrated by immuno-electrophoresis that one allergen was present in the glove extract, but not in NRL. In general, allergen patterns are notably simpler in glove extracts than in NRL, but it should be kept in mind that IgE-binding proteins detected in immunoblotting after sodium dodecyl sulfate polyacrylamide gel electrophoresis may also be split-down products of larger stem molecules. Information on molecularly characterized NRL allergens that

have been demonstrated in gloves is currently still very limited. Most of the IgE-binding capacity of one highly allergenic glove was attributable to hevein in a recent study (Alenius *et al.*, 1996a), and both REF and a 23 kDa protein have been identified in certain glove brands (Czuppon *et al.*, 1993; Lu *et al.*, 1995).

An attempt can be made to measure the "total" allergenicity of manufactured NRL products *in vivo* by specific IgE inhibition assays. In 1988, Turjanmaa *et al.* (1988a) use SPT to study the allergenicity of 19 surgical and household NRL gloves and found great variation among them. Later, Yunginger *et al.* (1994) studied 71 glove brands by RAST inhibition, and similarly demonstrated great variation (more than 3000-fold differences) in their allergen contents. Overall, the NRL gloves, especially the powdered ones, contained higher levels of extractable allergens than the other rubber products tested. In 1994, the Finnish National Research and Development Centre for Welfare and Health conducted a study of 20 brands of internationally sold surgical and examination gloves covering over 90% of the Finnish medical glove market. The allergenicity of glove extracts was assessed by three methods: SPT, RAST inhibition, and ELISA inhibition. Highly significant correlations ($r = 0.94$-0.96) between the methods were observed, indicated that the ELISA method can also be used for reliable NRL allergen quantification (Turjanmaa *et al.* 1996b). On the basis of these results, NRL gloves could be divided into three groups containing low (<10 arbitrary units [AU] per ml, nine gloves), moderate (10-100 AU/ml, three gloves), or high (>100 AU/ml, eight gloves) levels of NRL allergens. Both powdered and non-powdered brands were found among gloves with low allergen content. It is well established that cornstarch glove powder can

adsorb NRL allergens from the gloves (Tomazic *et al.*, 1994; Turjanmaa, 1994; Beezhold and Beck, 1992), and air-sampling studies have shown that NRL aeroallergen concentrations are high in areas where powdered "highly allergenic" gloves are frequently used (Swanson *et al.*, 1994; Tarlo *et al.*, 1994). At present, however, it is not known which of the main NRL allergens contaminate the glove powder.

2.6 T-cell Responses to Latex Allergens

Exposure to latex allergen results in the production of mainly IgE antibody in allergic patients (Breiteneder, 1998; Fink and Kelly, 1994). The present of IgE and Th 2 cytokines in allergic patients indicated that the immune mechanism is T-cell mediated. This was further substantiated by the results obtained in murine model of latex allergy (Thakker *et al.*, 1999; Xia *et al.*, 1999). However, not much attention has been paid to studying lymphocyte responses in latex-allergic patients or to elucidating the immunopathogenesis of latex allergy. The results of the few studies indicate that the crude antigens, such as ammoniated or non-ammoniated latex sap extracts obtained from finished latex products used in demonstrating the antibody responses, are toxic to lymphocytes (Johnson *et al.*, 1999).

When purified antigens were used, significant T-cell stimulation has been demonstrated. HPLC purified fractions with a molecular size of 3-10 kDa demonstrate PBMC (Peripheral blood mononuclear cell) stimulation *in vitro* (Turjanmaa *et al.*, 1989). Significant stimulation of PBMC from health care workers with latex allergy has been detected with a 30 kDa latex protein purified by HPLC and Sephadex G-200 chromatography (Murali *et al.*, 1994). Although

there was no correlation with latex allergens, there was no stimulation with this latex antigen when cells from normal subjects were studied. Raulf-Heimsoth *et al.* (1996) evaluated latex glove extract, latex sap and Hev b 1 in PBMC stimulation and obtained 47.8%, 65% and 52% stimulation, respectively, in latex allergic patients, compared to only 25%, 37% and 25% in non-allergic subjects exposed to latex allergens. There was, however, no correlation between specific IgE levels and the PBMC stimulation with these allergens (Raulf-Heimsoth *et al.*, 1996; Johnson *et al.*, 1999).

Johnson *et al.* (1999) using six purified latex allergens demonstrated significant PBMC stimulation with some of the latex allergens. Of the 28 HCWs studied, 12 failed to stimulate the PBMC with any of the six antigens, while the remaining 16 patients showed considerable variation in their responses. Hev b 2 stimulated the PBMC of all the remaining 16 patients, followed by Hev b 6, which stimulated the PBMC of 75% of patients. The other purified allergens tested showed less reactivity. Crude latex extract (MNA) showed the least response of the allergens. This study also failed to demonstrate any correlation with IgE binding and T-cell stimulation. Recently, significant T-cell stimulation was demonstrated with Hev b 5 in 5/6 HCWs with latex allergy (De Silva *et al.*, 2000). The two major epitopes identified in the stimulation of T-cell clones and lines include the peptides with amino acids 46-65 and 109-128. A predominantly T_H 2-type (T helper cell type 2) response was detected by the two peptides, as shown by the IL-5, but not IFN-γ, secretion by the two T-cell lines stimulated with the peptides (De Silva *et al.*, 2000).

2.7 Epitopes of latex allergens

The specificity of the allergens binding to IgE or to T-cells depends on the amino acid sequence of the allergens. Information on epitopes and their interaction with receptors may be immense value in devising specific immunodiagnostic and immunotherapeutic reagents for more reliable diagnosis and patient care. The T-cell epitopes are linear and MHC class 2 restricted, while the B-cell epitopes are usually conformational, and not MHC controlled. However, some of the linear B-cell epitopes may adequately block IgE binding and cross-linking of allergens on the surface of basophils and mast cells. Beezhold *et al.* (1997) detected six IgE-binding epitopes spanning the whole sequence of the prohevein molecule, of which four were in the C-domain of the Hev b 6 molecule. The N-terminal epitope had 100% homology with wheat-germ agglutinins and other plant proteins belonging to the chitinase family (Shinshi *et al.*, 1987).

On evaluation of the amino acid sequence of the Hev b 6 molecule with overlapping synthetic peptides, 10 IgE-binding regions were identified (Banerjee *et al.*, 1997). Some of these epitopes were specific for IgE binding to spina bifida patients, while the other showed specificity to HCWs patients. In addition, epitopes binding equally to the IgE of both patients have also been detected. The two N-terminal epitopes showed similar sequence homology with the WIN-1 and WIN-2 proteins of potato (Broekaert *et al.*, 1990; Lee *et al.*, 1991; Banerjee *et al.*, 1997).

The IgE-binding epitopes of Hev b 1 and 3 were studied with sera from both HCWs and SB patients with latex allergy (Kanitpong *et al.*, 2000). Hev b 1

showed eight IgE-binding epitope regions when SB patients were studied, while Hev b 3 showed 11 IgE-binding epitopes. Three epitopes from Hev b 1 and two from Hev b 3 reacted similarly with both groups of patients. The similar binding features noted with Hev b 1 and Hev b 3 allergens may be due to the present of similar epitopes and sequence homology. Of the eight epitopes identified in Hev b 1, six were in the N-terminal end, while only two were located in the C-terminal end. Hev b 3 showed 11 epitopes binding to IgE, of which the four N-terminal epitopes spanning amino acids 1-114 were highly conserved. All eight epitopes from Hev b 1 reacted with SB sera, but only three showed binding with sera from HCWs. It is interesting to note that amino acid 16-25 at the N-terminal end was specific for SB patients, while the two epitopes at the C-terminal end of Hev b 3 reacted with both HCW and SB patients. The epitopes at the N-terminal end of Hev b 3 were specific to SB patients.

By spleen cell stimulation in a murine model of latex allergy, Slater *et al.* (1999) identified four T-cell binding regions in Hev b 5, a proline-rich acidic protein of latex, showed four major IgE-binding regions representing amino acid 1-38, 55-74, 109-128 and 132-151. Two of the four T-cell epitopes failed to bind IgE, suggesting the usefulness of the sequence as an immunotherapeutic peptide. After study of the T-cell response in six latex-allergic patients, two immunodominant regions were identified in Hev b 5 (De Silva *et al.*, 2000). On careful evaluation of the epitopes, it was found that certain specific amino acids were highly conserved and the sequences of the epitopes were represented by KXEE or KEXE, where “X” is empty or represented by threonine or alanine (Slater *et al.*, 1999).

2.8 Latex Allergen Cross-reactivity

Since the first report suggesting allergen cross-reactivity between NRL and banana (M' Raihi *et al.*, 1991), a number of studies dealing with possible cross-reactivity between NRL and various food allergens have been published (Makinen-Kiljunen, 1994; Lavaud *et al.*, 1995; Ross *et al.*, 1992; Rodriguez *et al.*, 1993; De Corres *et al.*, 1993; Blanco *et al.*, 1994; Ahlroth *et al.*, 1995; Baur *et al.*, 1995; Alenius *et al.*, 1996c). RAST inhibition and immunospot and immunoblot inhibition have been used to verify cross-reacting IgE antibodies to latex and banana (Makinen-Kiljunen, 1994; Alenius *et al.*, 1996c), but 100% inhibition has not been observed in any experiment, indicating that only a few of the NRL and banana allergens are cross-reactive. The presence of at least one common allegen in NRL and banana has been verified by immunoelectrophoresis (Makinen-Kiljunen, 1994). In addition, there is no evidence that sensitization to banana may result in the production of IgE antibodies which cross-react with allergens in native NRL, but not with allergens in gloves (Makinen-Kiljunen, 1994). Allergens in native NRL may be denatured during the storage of NRL in ammonia or be otherwise modified during glove manufacture.

Immunoblot inhibition has demonstrated cross-reacting IgE antibodies to several proteins in NRL and banana (Alenius *et al.*, 1996c). Lavaud *et al.* (1995) and Vallier *et al.* (1995) have suggested that 15 and 30 kDa proteins are important cross-reacting allergens in NRL and banana. One 15 kDa protein is prolifin, an actin-regulating protein, shown to be an important cross-reacting allergen of several plant sources such as tree, grass and weed pollens and fresh

fruits and vegetables (Valenta *et al.*, 1992). Prolifin has been demonstrated in NRL and banana, but not in NRL glove extracts (Vallier *et al.*, 1995). Latex RAST positivity was not correlated with birch, mugwort, or timothy pollen RAST (Makinen-Kiljunen, 1994), but cross-reactivity has been reported among NRL, ragweed and blue grass allergens (Appleyard *et al.*, 1994).

About half of the NRL-allergic patients have experienced symptoms after eating banana and 35% have had positive skin test results to fresh banana (Makinen-Kiljunen, 1994; Blanco *et al.*, 1994). Among 47 NRL-allergic patients, 26 (55%) had IgE antibodies also to banana and of the 31 latex RAST-positive patients, 25 (81%) also had positive banana RAST (Makinen-Kiljunen, 1994). Besides banana, other foods, such as avocado, chestnut, tomato, kiwi, melon, pineapple, peach and papaya have been though to cross-react with NRL.

Objective

The objectives of this study are:

1. To study the protein composition of bottom fraction membrane (BFM) from fresh latex of *Hevea brasiliensis.*
2. To study the allergenicity of BFM proteins.
3. To study the alkaline stability of BFM proteins VS others in fractionated fresh latex or different native compartmental sites.
4. To study changes of field latex composition upon processing into latex concentrate.
5. To study the protein composition of latex concentrate with respect to BFM protein contamination.
6. To study extractable proteins in NR latex gloves and indirectly identify native compartmental sites in fresh latex.