Chapter 1

Introduction

For nearly sixty years natural latex has been used in the manufacture of many household products such as rubber teat, condom, gloves, urinary catheters without even the slightest hint that any of its 'natural' components could cause any sort of problem. It is clear for a long time that repetitive skin contact with rubber products containing high accelerator or antioxidant residues, if maintained for sufficient time, may sensitize many people. Serious skin problems can subsequently develop and progressively worsen if contact with an allergenic rubber latex product is continued (Gonzalez, 1992). The severe allergic reaction (type I) to latex was first reported in Germany in 1927. In 1993, the US Food and Drug Administration (FDA) received over 1,100 reports of injury and 15 of deaths associated with these latex allergy (Slater, 1993). Recent reports on widespread of these life-threatening latex-associated allergies have focused attention on latex proteins as serious allergens. Current estimates derived from analysis of sera from blood donors indicated that up to 6.5 % of the U.S. population at present may be hypersensitive (Ownby, et al., 1996).

1.1 Background and literature review

Natural rubber tree, *Hevea brasiliensis* , is a native plant of Central and South America and the Caribbean basin. In 1876, rubber seeds gathered from the Amazon valley were planted in Kew Gardens, London, and about 2,800 of the seeds germinated and the rubber plants were shipped to Sri Lanka in 1877. A few plants were sent from Sri Lanka to Malaysia and from there, in 1882, seeds and plants were sent to

Singapore, India and Indonesia. Six years later (1888), the first practical pneumatic tire was developed, eventually led to an unprecedented demand for natural rubber (St.Cyr, 1984). The latex producing rubber trees grow to the heights of 15-20 m and require 2,000-2,500 mm/y of rainfall. They thrive at altitudes up to 300 m (Edgar, 1958). Trees are commonly planted 3-4 m apart in parallel rows 6 m apart. Experiments are being conducted to determine the feasibility of higher planting densities. It takes up to 7 years for a rubber tree to grow to maturity. The time of immaturity in the field can be reduced to 5 years by growing the trees in plastic bags in nurseries for 1-1.5 years before transplanting to the field (St.Cyr, 1984).

Rubber trees can be propagated either by sexual method or by vegetative methods. In normal practice, the trees are propagated by grafting buds from a mother tree onto the stem of a seedling. All trees derived by this method from a single mother tree either directly or indirectly are called a clone (St.Cyr, 1984). The mother tree is chosen for the best combination of a variety of properties, including yield, resistance to disease, resistance to wind damage, bark characteristics, and various growth characteristics. Today there are less than 100 clones that are commercially grown for latex production, but many times that number have been grown experimentally. Clones RRIM600, GT1 and PB5/51 had been highly promoted to plant in Thailand since 1972. Today the clones that are recommended to plant in Thailand by the Rubber Research Institute of Thailand are varied for each region, and clones BPM24, RRIM600, GT1, PR255 and PR261 are the first clones of choice. Rubber trees are subjected to a variety of diseases but the most devastating is the South American Leaf Blight caused by the fungus *Microcyclus ulei* (St.Cyr, 1984), which retarded extensive cultivation in South and Central America. At present, the total world's rubber plantation areas are about 9,759,800 Hectares (ha). There are about 8,941,600 ha in

Asia and Pacific, especially in the South East Asia countries, they constitute about 80 % of the rubber planting areas of the world (7,803,800 ha). In Africa, there are about 459,900 ha and in South America there are 358,300 ha of rubber plantation areas (Rubber Statistical Bulletin, Jan. 1997).

Rubber trees were first planted in Thailand in 1901 by Praya Rachadanupadit Mahidsorn Prakdee (Ko-simbee) in Trang Province. Up to now, rubber plantations are distributed nearly all over the country. The data from Rubber Statistical Bulletin (1997) shows that Thailand is the largest natural rubber producer in the world. Natural rubber production in 1995 was about 1,784,400 metric tons while the world production was 5,880,000 metric tons (Figure 1.1). There are about 1,949,000 ha for rubber plant cultivation and there are more than 10 million people earning their livings with this plant. Most of the natural rubber produced is exported, only small fraction (about 8%) is consumed in the country. The important natural rubber productrs in Thailand are tires (50% of domestic consumption), elastic rubber thread, sport and toys, shoes, gloves, condom and automotive parts. There are 138 rubber gloves-factories, registered, that can produce 5,600 million pair of gloves per year and two condom-factories that produced 240 million pièces each year (Tasakorn, 1996). There are more than 5 million people in Thailand who are in direct contact with latex or latex products, in two aspects, i.e. the producers and the users or consumers.

1.2 Natural rubber

Natural rubber (NR) is a coagulated product of a complex cytosolic mixture obtained mainly from the rubber tree *Hevea brasiliensis*. This cytosolic fluid or latex is generally milky in appearance, which flows from the plant after the slightest wound. It is produced by a highly anastomosed system of cells and then stored in tubular

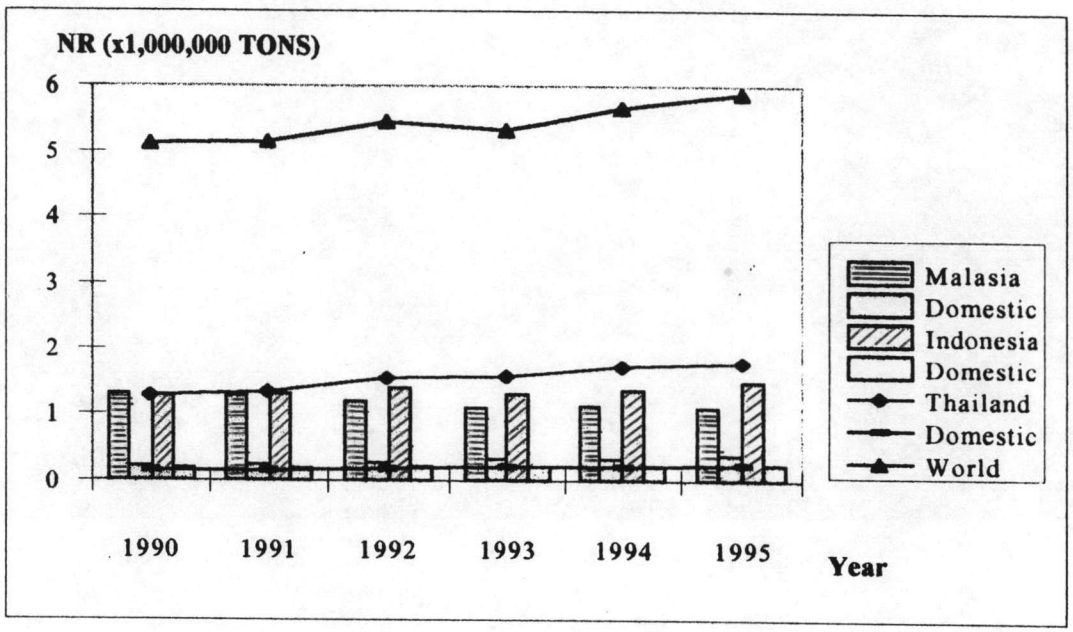

Figure 1.1 World natural rubber production and major rubber producers. The major rubber producer are Thailand, Indonesia and Malaysia.

Figure 1.2 Latex rubber tapping: farmer uses the tapping knife to cut remove shaving of bark from the surface of a groove into the tree in the early morning.

structures known as laticifers. The laticiferus cells synthesize rubber molecules by many steps of biochemical reactions as summarized in Figure 1.3. The major component of a natural rubber molecule is *cis*-1, 4-polyisoprene that usually constitutes to 25 to 35 % dry matter in fresh field latex. Approximately 7,000 other species of plants produce latex containing polyisoprene rubber, but only *H. brasiliensis* lattices are used to produce rubber in commercially significant quantities. The fresh natural rubber latex (NRL) is collected from the tree by a process called tapping, which has been described as a controlled wounding of the tree (St.Cyr, 1984). A specially designed tapping knife is used to remove the bark from the surface of a groove made into the tree to a depth about 1 cm from the cambium. The groove is made from left to right at an angle about 30^{o} to the horizontal across half the tree (Figure 1.2). The latex exudes onto the surface of the cut and flows down the cut into the collection cup. Coagulation of the latex will occurred naturally, in order to prevent it, a small amount of a preservative, usually ammonia, sodium sulfite, formaldehyde, or boric acid, is added to the latex. Field latex or freshly tapped NRL contains the total solids of 25-40% (w/w) and the dry rubber content is usually about 3 %(w/w) less than the total solids content (TSC). The 3 %(w/w) non-rubber portion is made up mainly of protein, lipid and sugars. When the latex is centrifuged, it is separated into three phases: (1) a white supernatant fraction, that is a colloidal solution of rubber particles, (2) an aqueous phase which is called clear serum(C-serum), is corresponding to the latex cytosol, and (3) a yellow bottom fraction consists essentially of organelles, Frey-Wyssling particles and lutoids.

The field latex is not utilized in its original form due to its high 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 per cent or more of rubber (Gazeley et

al., 1988). Latex concentrate is differentiated by the method of concentration and type of preservative used. There are specialty forms of latex concentrate differ from the general purpose type of latex such as double centrifuged latex, creamed/centrifuged latex and modified types such as prevulcanized latex. Three methods of concentration are employed, centrifugation, evaporation and creaming; centrifugation is the preferred method and accounts for 95 per cent of total production. The preservation systems used in centrifuged concentrate are: 0.7% ammonia for high or full ammonia (HA); 0.2 % ammonia, 0.025% zinc oxide, 0.025% tetramethylthiuram disulphide for low ammonia TZ (LA-TZ); 0.2% ammonia, 0.2% sodium pentachlorophenate, for low ammonia pentachlorophenate (LA-SPP); and 0.2% ammonia, 0.24% boric acid for low ammonia boric acid (LA-BA). Latex concentrate prepared by evaporation is usually stabilized by potassium hydroxide, while creamed latex is normally preserved with 0.7 % ammonia.

1.2.1 Natural rubber tree

The plants that containing latex belong to several different families but are mainly of Dicotyledons (Metcalfe, 1967), only a few species are cultivated and have economic importance (Bonner and Galstone, 1943) . Among of them, *Hevea brasiliensis* is the best rubber producer tree. This tree, which grows in the hot humid intertropical regions, processes articulated laticifers in the bark, although it is thought that laticifers are also capable of apical intrusive growth in the cotyledons, inner seed coats, and in young leaves (Fay and Jacob, 1989). The first laticifers appear at the junction between the cotyledons and the axis of the hypocotyl, and develop towards the base of the hypocotyl or towards the top of the plumule (Gomez, 1982). Laticifers form from procambial cells in young plantlets, they are found in the primary phloem in shoots,

Table 1.1 Taxonomy of the rubber tree.

Division	Spermatophyta
Sub-division	Pteropsida
Class	Angrosperm
Sub-class	Dicotyledon
Order	Euphorbiales
Family	Euphorbiaceae
Genus	Hevea
Species	brasiliensis

roots, the veins of young leaves, flowers and fruits. At the same time that cambium has formed it produces a special laticiferous system in the secondary phloem. Articulated, anastomosing laticiferous vessels form successive vertical networks called rings or mantles. In *Hevea* these secondary laticiferous vessels 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 several times.

The laticiferous system of *Hevea* has been studies and show that it's functions related with others surrounding tissues. Trunk tissue is derived from the functioning of lateral meristem, the vascular cambium which forms secondary xylem and phloem. The following details had been studies: (1) primary tissue formed by the shoot apical meristem before the cambium becomes active; pith, primary vascular tissue, and cortical tissue and (2) tissue driven from the functioning of other lateral meristem; cork cambium. In bark which has not yet been tapped the latter protective tissue or periderm consists mainly of several outer layers of cork cambium commonly called cork(Figure 1.3).

Wood, formed in the trunk by the cambium, is a strong material which conserves its basic structure even if the living elements it contains degenerate or die at a depth. In contrast, the phloem, which forms towards the outside of the trunk is fragile tissue which is modified continuously. In particular, its structure must adapt to the increased girth of the trunk, and the laticifers exploited for rubber production are differentiated in the phloem. The articulated laticiferous vessels are thus arranged in concentric rings in the phloem. The contiguous walls of two adjacent laticifers become perforated in several places producing anastomoses which create a continuous network within each mantle. Inside the network, the laticiferous vessels are inclined slightly either to the

right or in an undefined manner. The existence of anastomoses between rings has long been a subject of controversy.

The phloem also contains cell other than laticifers, including sieve tubes and companion cells. In *Hevea brasiliensis,* sieve tubes are formed of long cell elements connected at the ends by their inclined end walls, which are transformed into compound sieve plates with numerous pores. Only the newly formed sieve tubes (i.e., those nearest to the cambium) are functional. They form a narrow strip of conducting phloem in the deepest part of the phloem. The thickness of this band varies on average from 0.2 to 1 mm in adult trees (Hebant and Fay, 1980). Outside this zone the sieve plates of sieve elements may be blocked by massive deposits of definitive callose. They are then soon deformed and even crushed under the pressure of the living tissue around them. The callose then dissolves. The sieve tubes in most of the phloem are thus not functional. The phloem also contains vertical rows of parenchyma cells; some of them are closely associated with the laticifers, each ring being enveloped by a sheath of parenchyma cells. Other parenchyma cells, grouped in horizontal radial lines forming hextero-cellular and multiseriate phloem rays with procumbent and upright ray cells, are also found in the phloem. These continue through the cambium towards the wood.

Many of these parenchymatous cells process vacuoles containing tannin. In untapped bark, the tannin cells are mainly arranged around the laticifers, whereas in tapped bark they are more numerous in the vascular rays. In young *Hevea*, this preferential location of tannin is confirmed in the cells adjoining the mantles, and which in fact form the parenchymatous sheath for the laticifers.

The wood is connected to latex-producing phloem by the vascular rays. Its role in the translocation and distribution of xylem sap and in the formation of the tree's

organic reserves means that it participates actively in growth-production balances. A structural study of *Hevea* sap wood was carried out by Henon in 1981.

It is difficult to observe growth rings in the trunk, but it is known that rhythmic growth rings exist in young wood. In adult *Hevea*, wood growth in the trunk occurs for most of the year but stops when leaves are renewed and during flowering (Fay and Jacob, 1989).

The distribution of plasmodesmata in tissue or an organ may contribute to the establishment of local translocation pathways for sugar and water. This type of study was carried out on leaves, particularly in maize. In *Hevea*, the high number of plasmodesmata between the parenchyma cells of the vascular rays and the high enzymatic (acid phosphatases, ATPases) and respiratory activities of these cells argue in favor of the transport of sugars in the symplast (cytoplasmic continuum). This translocation, as far as the parenchyma cells adjacent to the laticifers, could be carried out by the symplast, since there are numerous plasmodesmata between all these parenchyma cells. However, it has not been ruled out that sucrose may be translocated by the apoplast, as is though to be the case in certain species. The absence of plasmodesmata in mature laticifers implies that nutrients must penetrate by crossing laticifer walls (apoplast) and laticifer plasmalemma. The water which thus enters the laticifers may come from the neighboring parenchyma cells. In addition, the fall in turgor pressure in the laticifers, and in all the phloem cells in the trunk during the day, and hence their loss of water, is explained by the transpiration phenomena (Buttery and Boatman, 1966).

The functional organization model of the secretion tissue of *Hevea brasiliensis* implies the existence of complex interrelations between vertical and horizontal translocation. Vascular rays are the horizontal transport road for metabolites flowing to

the laticifers and which come either from the underlying wood or from functional sieve tubes. In addition to this short distance transport there is the long-distance vertical transport phenomena has been studied in detail in other plant (Zimmermann and Miburn, 1975). In exploited *Hevea*, the important role of the parenchymatous elements (in ray cells and in vertical sheathing parenchyma) in loading laticifers is stressed. It thus appears that laticifer metabolism must depend on the satisfactory functioning of other cells in the secondary tissue in the trunk.

Stimulation of *Hevea* is now common practice in plantation. However, use of the most effective stimulants, hormones, 2,4,5-T, 2,4-D, and ethylene, has secondary effects on *Hevea* bark. The first phenomenon revealed was the transformation of bark under the effect of 2,4,5-T and 2,4-D. Monthly application of 1% active ingredient caused thickening of regenerated bark whether the substances were applied to freshly cut bark or below the cut. In addition, regeneration was very irregular after direct application, and the bark flaked easily. Histologically, the outer parts of regenerated bark displayed masses of parenchyma-like tissue. The number of laticiferous mantles did not increase. These observations lead to supposing that the hormonal substances increase the formation of periderm material by activating the subero-phellodermic layer. However, they probably have no effect on the vascular cambium (Gomez, 1964, Jonge, 1955 and 1957).

The effects of hormonal stimulants on virgin bark in the tapping panel have not yet been studied at agronomic concentrations. However, over-intensive treatment with 2,4-D and Ethrel (ethylene generator), carried out evaluate the effect of stimulation on the brown bast phenomenon (Fay and Jacob, 1989), revealed that the abnormal thickening of stimulated bark, its spongy appearance, necrotic areas, small local cracks, and the appearance of nonproductive zones along the tapping cut were all

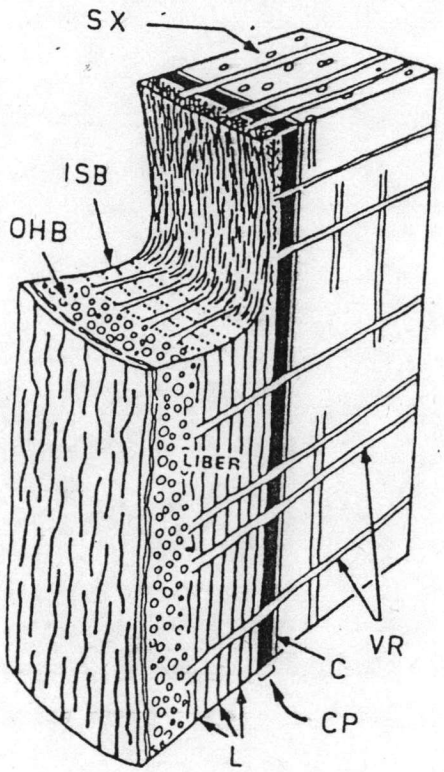

Figure 1.3 The general organization of *Hevea brasiliensis* bark at tapping cut level. C = cambium; CP = conducting phloem; ISB = inner soft bark; L = laticiferous vessels; OHB = outer hard bark; SX = secondary xylem; VR = vascular ray. (From Fay, E.dc, Jacop, J-L. Anatonical organization of the laticiferous system in the bark : Physiology of rubber tree latex. CRC Press, Inc.Florida USA. 1989 ; 6)

connected with an increase in cell division in the parenchymatous sheaths surrounding the rings of laticifers. In addition, ethylene can have necrotic effects in the laticifers themselves which can render them nonfunctional.

In poor soil the rate of laticifer mantle formation probably falls to 1.74 per annum instead of 3.14 in rich soil (Fay and Jacob, 1989). In addition, application of fertilizer modifiers the quantity of laticifers and aclereids cells and regeneration of bark (Pushparajah, 1969, Samsidar et al., 1976). It is thus clear that the minerals available in the soil affect bark anatomy. The effects of mineral deficiencies have been studied with precision in young *Hevea* plants. It was seen that N,P,K, and Mg deficiency reduced stem diameter, bark and phloem thickness, cell size, and the number and size of laticiferous vessels. The laticifer index proposed to report on the productivity of *Hevea* bark (average number of laticifer per unit of surface area multiplied by the average conductive area of a laticifer and the average diameter of the stem) was found to be significantly lower for plants deficient in N, P, K, Mg, Ca, S, Mn, and Zn.

1.2.2 Rubber synthesis

Rubber biosynthesis can be divided into three stages: (1) generation of acetyl-CoA, (2) conversion of acetyl-CoA to isopentenyl diphosphate and (3) polymerisation of isopentenyl diphosphate units to rubber. The first two stages are located in the soluble phase of the latex, while the third stage occurs only at the surface of the rubber particles (Figure 1.3). The latex serum contains all the enzymes of the glycolytic pathway and the isopentenyl diphosphate synthesis from acetyl-CoA. The conversion of acetyl-CoA to isopentenyl diphosphate follows the terpenoid pathway used by plants for the synthesis of sterols and carotenoids (Figure 1.4). Two acetyl-CoA first condense to form acetoacetyl-CoA, then condense with the another acetyl-CoA to

form 3-hydroxy 3-methylglutaryl-CoA. This six-carbon intermediate then loses its CoA and is reduced to mevalonic acid using NADPH, which is generated by the pentose phosphate pathway. The conversion of mevalonic acid to isopentenyl diphosphate occurs via the formation of mevalonate 5′ phosphate and mevalonate 5′ diphosphate (Figure 1.4). An isomerisation of isopentenyl diphosphate to dimethylallyl diphosphate is catalyzed by isopentenyl diphosphate isomerase (Figure 1.3), occurred before the polymerisation takes place by the successive additions of isopentenyl diphosphate to dimethylallyl diphosphate to form, geranyl diphosphate (C_{10}), farnesyl diphosphate (C_{15}) and geranylgeranyl diphosphate (C_{20}), respectively as shown in Figure 1.5. The initial linking of isoprenyl units occurs with the *trans*-configuration. The subsequent repeated addition of isoprenyl units occurs in *cis*-configuration to give rise to the bulk of the 30,000 isprenyl units of the final rubber molecules. In the latex serum numerous enzymes have been reported. These functional proteins play many important roles in biological survival of the rubber plant.

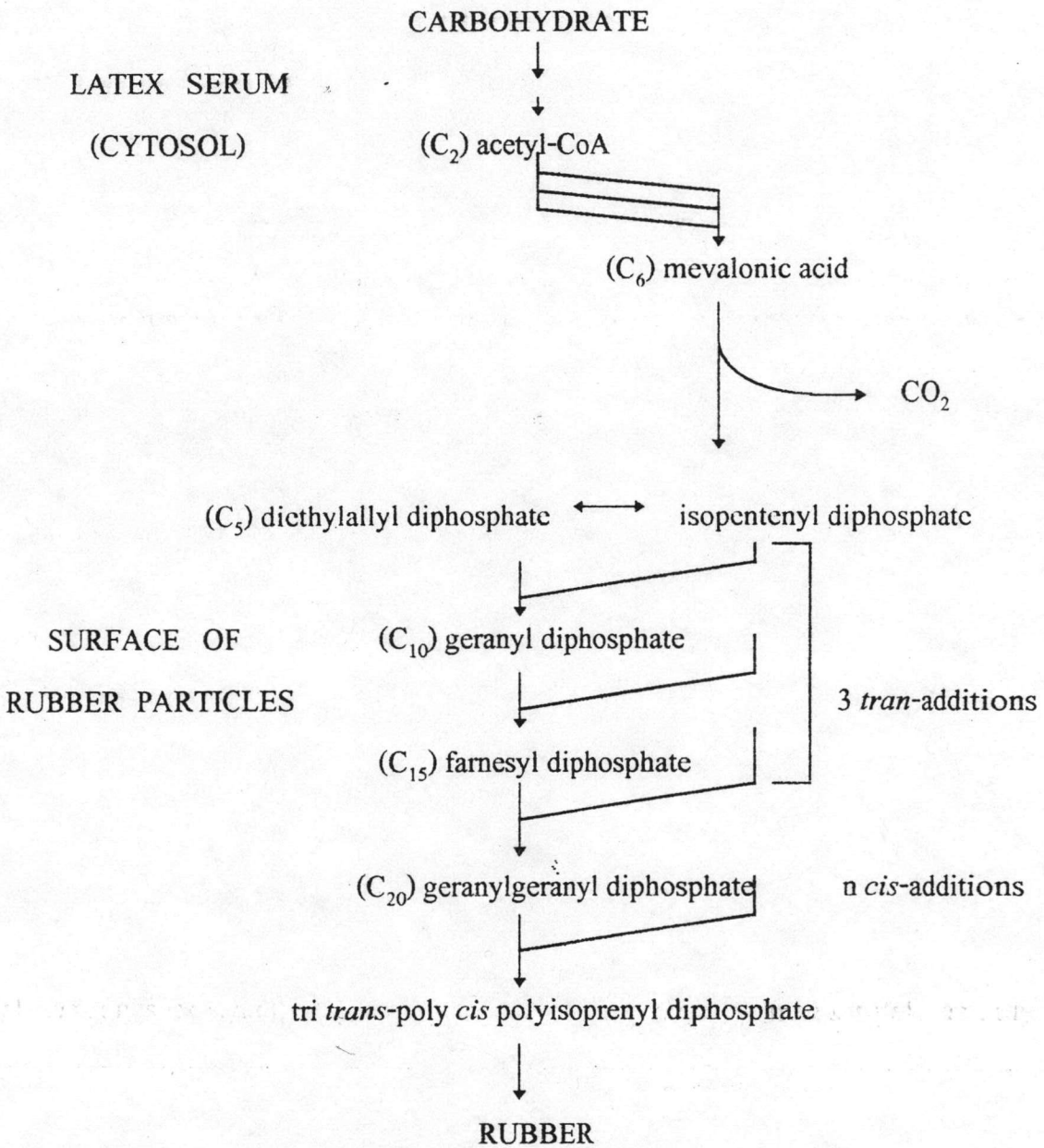

Figure 1.4 Outline of rubber biosynthesis in the latex of the rubber tree.

(From: John, P. Biosynthesis of the major crop products.

John Wiley & Sons: 1992).

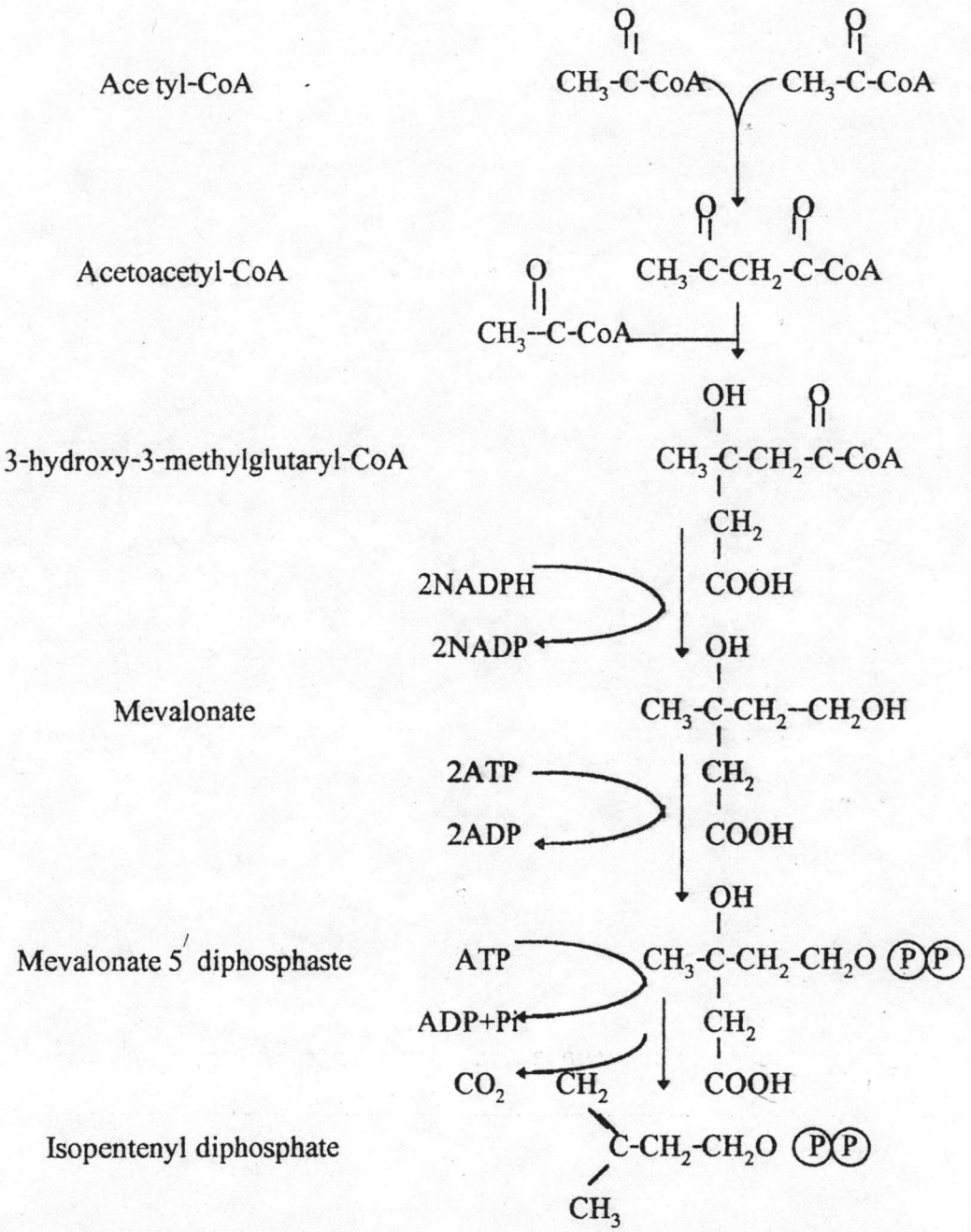

Figure 1.5 Formation of isopentenyl diphosphate from acetyl-CoA in rubber biosynthesis. (From: John, P. Biosynthesis of the major crop products. John Wiley & Sons: 1992).

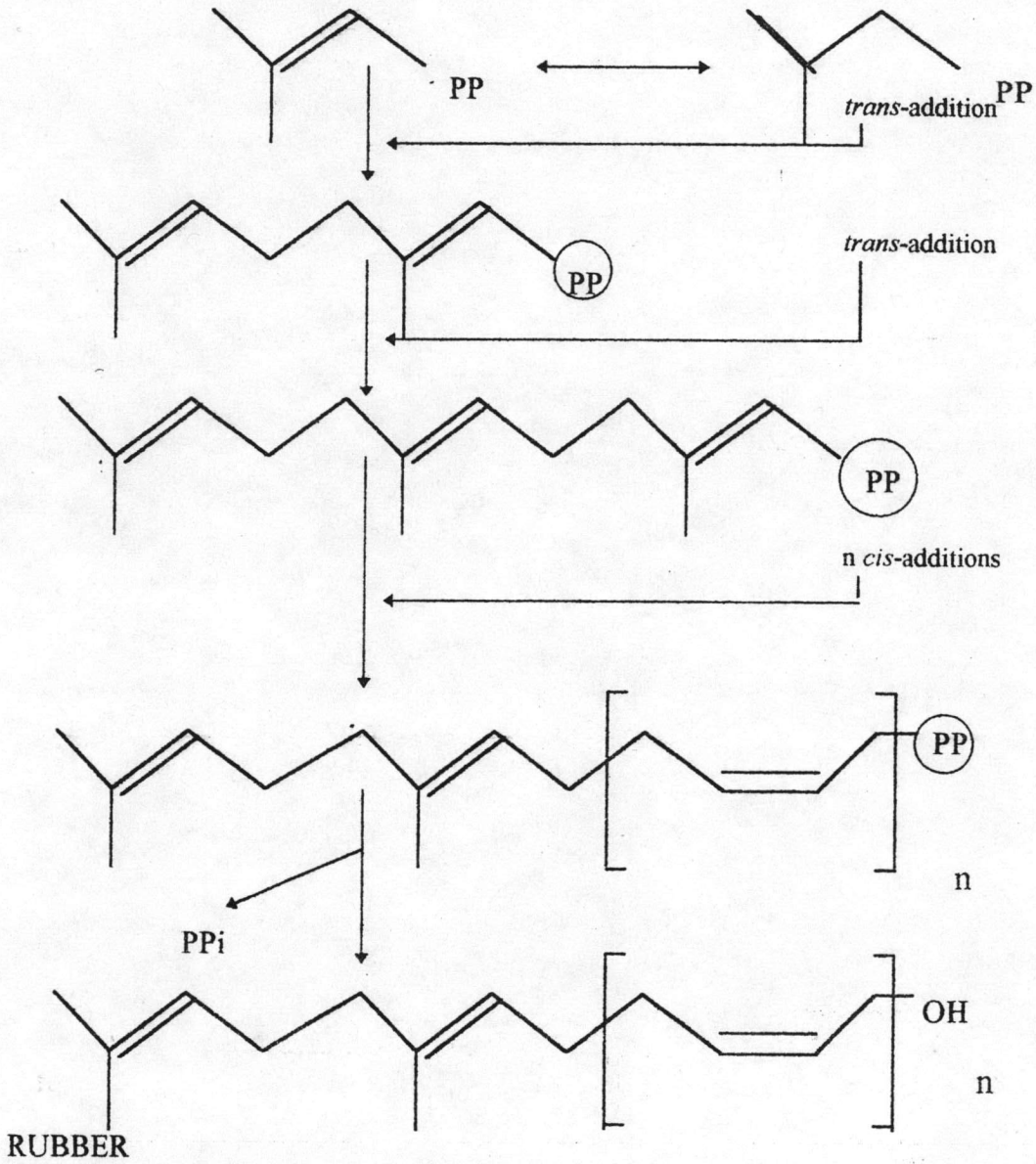

Figure 1.6 Polymerisation of isopentenyl diphosphate to form rubber. (From: John, P. Biosynthesis of the major crop products. John Wiley & Sons: 1992).

1.2.3 Rubber proteins

Proteins in fresh latex are distributed in three phases. In rubber particle phase, proteins or polypeptides associated with the rubber particle phase are about 26% of the total proteins in the latex. The rubber particle has been studied and the data shows that it associates with the 14.6 kD protein which are rubber elongation factor (REF). The 24 kD protein and the other proteins ranging in molecular weight from 14-70 kD are also found associated with the rubber phase.

C-serum proteins has about 48 % of the total protein. About fifty different enzymes are distributed in this phase. Data from two dimensional immuno-electrophoresis shows the presence of about 30 components (Kekwick, 1993). Kekwick found that the predominant peak is a molecular weight of 80 kD that is composed of two 40 kD sub-units.

Bottom fraction is composed of the lutoid particles. There are about eight protein components, five cationic and three anionic, separable by electrophoresis, at pH 8.6 (Kekwick, 1993). Two of these proteins have been characterized. The most cationic, heveamine (Archer, 1976), is a lysozyme and the most anionic, hevein (Archer, 1960), has a molecular weight of 43 kD and contains chitin-binding domains similar to those found in a number of other plant species.

Analysis of the proteins in rubber particle of high ammonia latex (HA latex) shows the presence of a 12 kD polypeptide that is a proteolytic product of the 14.6 kD REF, some of which remains intact (Kekwick, 1993). The proteins of the HA latex C-serum do not resolve well on SDS-PAGE, but gel permeation chromatography on *Sephacryl S 300* removes material which causes smearing of the gels. The apparent molecular weight distribution of the protein of HA latex serum shows a peak in the far higher than fresh C serum or B serum, suggest that protein aggregation has occurred

during preservation and storage (Kekwick, 1993). The aggregation of both the serum 14.6 kD REF polypeptide and other proteins in HA latex serum was demonstrated by a comparison of the ELISA assays using specific antisera to the polypeptides of the fraction of HA latex and fresh C serum eluted from the *Sephacryl S 300* column (Kekwick, 1993).

The SDS-PAGE analysis of the proteins of commercial glove extracts of dipped manufacture shown a variation in polypeptides composition. Immunoblots using a specific antiserum to the REF failed to show its presence in any glove elute, although occasionally an ill-defined smear stretching about the length of the gel was obtained by Kekwick et al., (Kekwick, 1993). Extracts of some gloves, in Kekwick experiment, gave a positive reaction to the 40 kD dimer protein and its sub-unit as the 26 kD proteolytic fragment.

1.3 Allergy

Natural rubber latex products have been reported to cause delayed and immediate hypersensitivity. The usual delayed hypersensitivity reaction of eczematous contact dermatitis is caused by latex itself, and by chemicals added during rubber manufacture. These include accelerators to speed curing, namely mercapto-benzothiazole and derivatives, tetramethylthiurams and dithiocarbamates, and antioxidants such as *p*-phenylenediamine. Immediate hypersensitivity to latex are more serious because they involve not only the skin but mucosal surfaces. Manifestations within minutes may be contact urticaria, angioedema, rhinitis and respiratory symptoms, including dyspnea and asthma attacks. Patients subject to immediate reactions risk severe and fatal consequences if the hypersensitivity is unrecognized when they are examined or operated on by physicians or surgeons wearing latex gloves. Increased use of condoms, especially by those at risk of contracting HIV infection, further expose the population to latex hypersensitivity reactions.

1.3.1 Definition of allergy

The term allergy refer to certain diseases in which immune responses to environmental antigens cause tissue inflammation and organ dysfunction. The clinical features of each allergic disease reflect the immunologically induced inflammatory response in the organ or tissue involved. These features are generally independent of the chemical or physical properties of the antigen. The diversity of allergic responses arises from the involvement of different immunologic effector pathways, each of which generates a unique pattern of inflammation. The classification of allergic diseases is based on the type of immunologic mechanism involved. According to

Coombs and Gell's classification from 1963 (Gell and Coombs, 1974), allergic reactions can be divided into four types (type I-IV).

1.3.2 Allergens

An allergen is any antigen that causes allergy. The term is used to denote either the antigenic molecule itself or its source, such as pollen grain, animal dander, insect venom, food product or other natural products. Hypersensitivity and sensitivity are often used as synonyms for allergy. Immediate hypersensitivity and delayed hypersensitivity are the terms formerly used to define antibody-mediated allergy and T lymphocyte-mediated allergy, respectively.

1.3.3 Allergic classification

There are four types of allergic reaction (Gell and Coombs, 1974). Type I, Anaphylactic Type Hypersensitivity, a special class of antibody (cytotropic antibody, mainly IgE) binds to mast cells and basophils through the Fc fragment. When antigen reacts with these antibodies, vasoactive amines and other mediators are liberated and elicit the reaction. Type II, Cytotoxic Type Hypersensitivity, antigens on the cell surface combine with antibody. This may lead to opsonization and phagocytosis without complement, may facilitate attack by T cells, or may lead to binding of complement, which promotes immune adherence to phagocytes; or lytic effect may result in membrane by complement. Type III, Complex-Mediated Hypersensitivity, antigens combine with antibody to form complexes that in turn activate complement and Hageman factor (factor XII in blood coagulation) and aggregate platelets. Type IV, Cell-Mediated Hypersensitivity, T-lymphocytes carrying specific antigen receptors become activated by contacting with that antigen, proliferate, transform, and release a

variety of mediators (lymphokines) that in turn act on macrophages, lymphocytes, and other cells to yield the reactions of delayed type hypersensitivity.

1.3.4 Antibody molecules

Antibodies molecules are immunoglobulin and composed of four polypeptide chains, comprised of two identical copies of each of two nonidentical polypeptide chains, chains L and H giving $(LH)_2$. There are five classes of immunoglobulin, IgG, IgA, IgD, IgE, and IgM, depend on its H chain types. The H chain types are called γ in IgG, μ in IgM, α in IgA, δ in IgD and ε in IgE. In the most common immunoglobulin, IgG, the two H or heavy chains have approximately 440 amino acids (MW 50,000). The smaller L or light chains, always belong to one of two types: (kappa) and (lambda), contain about one-half the number of amino acids of the H chain (MW 25,000). The four polypeptide chains are covalently inter-connected by disulfide bonds.

1.3.5 The major immune cells

The immune system provides us with a most fascinating example of cell development in the mature vertebrate organism. A feature of the immune system is the constant generation of new cells that represent variations in gene organization of the immune system. The object of this variability is to provide the organism with immune cells that have the widest possible range of specificity. When the organism is invaded by foreign agents, the intrusion excites the immune system. Those immune cells that carry the specific immune receptors for interacting with the foreign agent are stimulated to proliferate. In a short while, usually a matter of days, clones of immune cells with the appropriate specificity have been produced and the organism fends off

the invader with the specific immunology tools provided by these cells. The clones of cells tend to persist for some time, which accounts for the fact that the second time the same foreign invader makes its presence known, it is rejected more promptly and without crisis.

There are two types of white blood cells or lymphocytes associated with the immune response; these are called B cell and T cell. B cells originate from precursor cells in the bone marrow and become implanted in peripheral lymphoid tissue, the spleen and lymph nodes. Each B cell has undergone genetic changes so that it has the potential for making a unique type of antibody protein. Its surface contains a limited number of highly specific receptors. The receptors are a special class of membrane-bound antibodies. When the organism is exposed to a specific foreign agent or antigen, the antigen reacts with B cells that carry complementary receptors. This contact stimulates proliferation of the original B cells (stem cells) as well as the further differentiation of some of them to make large quantities of antibodies (plasma cells). The plasma cells are considerably larger and contain a great deal of endoplasm reticule for the synthesis of specific antibody.

T cells also originate from stem cells in the marrow, but they mature in the thymus gland. They can also be stimulated to proliferate by exposure to an appropriate antigen but, unlike the B cells, their specific effect molecules remain firmly bound to the cellular membrane, as opposed to being secreted. Several types of T cells with different functions have been recognized. One class of T cells, known as T helper cells, promotes the maturation of antigen-stimulated B cells.

1.3.6 Antibody class switching

The antigen-binding specificity of an immunoglobulin molecule is determined by the NH_2 terminus of the immunoglobulin heavy and light chain, which is highly variable. By contrast, the COOH terminus of the heavy chain has constant amino acid sequence that determines the effector functions of the immunoglobulin molecule, such as binding to Fc receptors on different cell types and activation of secondary pathways. The variable region of immunoglobulin is encoded by multiple germ-line elements that are assembled into complete V(D)J variable regions during B-cell differentiation by a common enzymatic activity (recombinase), which is likely to be encoded by the RAG-1 and RAG-2 genes (Geha, 1992).

During an immune response, a B lymphocyte can express different heavy-chain isotypes sharing the same V(D)J region. This phenomenon (heavy chain class switching) allows a single B-cell clone to produce antibodies that retain variable region specificity in association with a different CH region gene, that is, with a different effector function. Class switching is believed to result from a recombination event that juxtaposes a downstream CH gene to the expressed V(D)J gene. Intervening sequences, including the previously expressed CH gene, are deleted. Recombination involves characteristic repetitive sequences (S regions) located 5′ of the C gene and corresponding S regions located immediately 5′ CH gene, except C. S regions are 2 to 10 kb in length, and their sequence consists of short tandem repeats (e.g., GAGCT and GGGCT). S region recombination sites are not flanked by obvious consensus within the S regions.

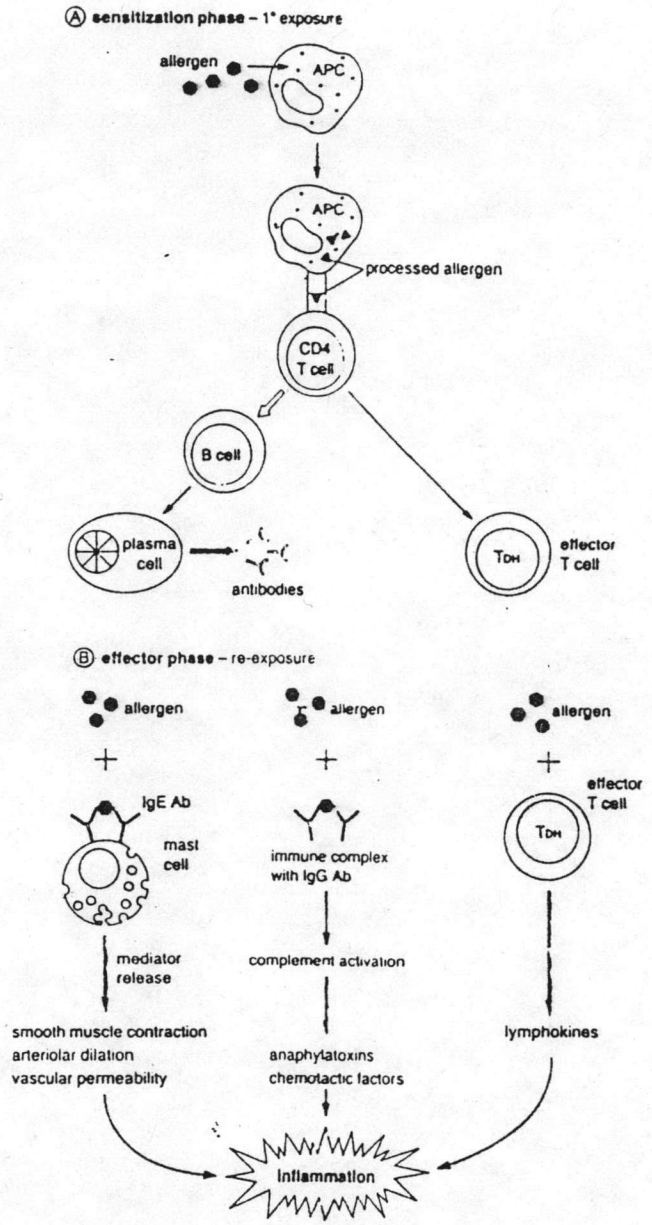

Figure 1.7 Role of the immune system in allergy. A: Sensitization phase, showing immunologic response to allergen from unsensitized (nonallergic) state to sensitized (allergic) state. B: Effector phase, showing reaction on reexposure of allergen to specific antibody or to specifically sensitized effector T cell.

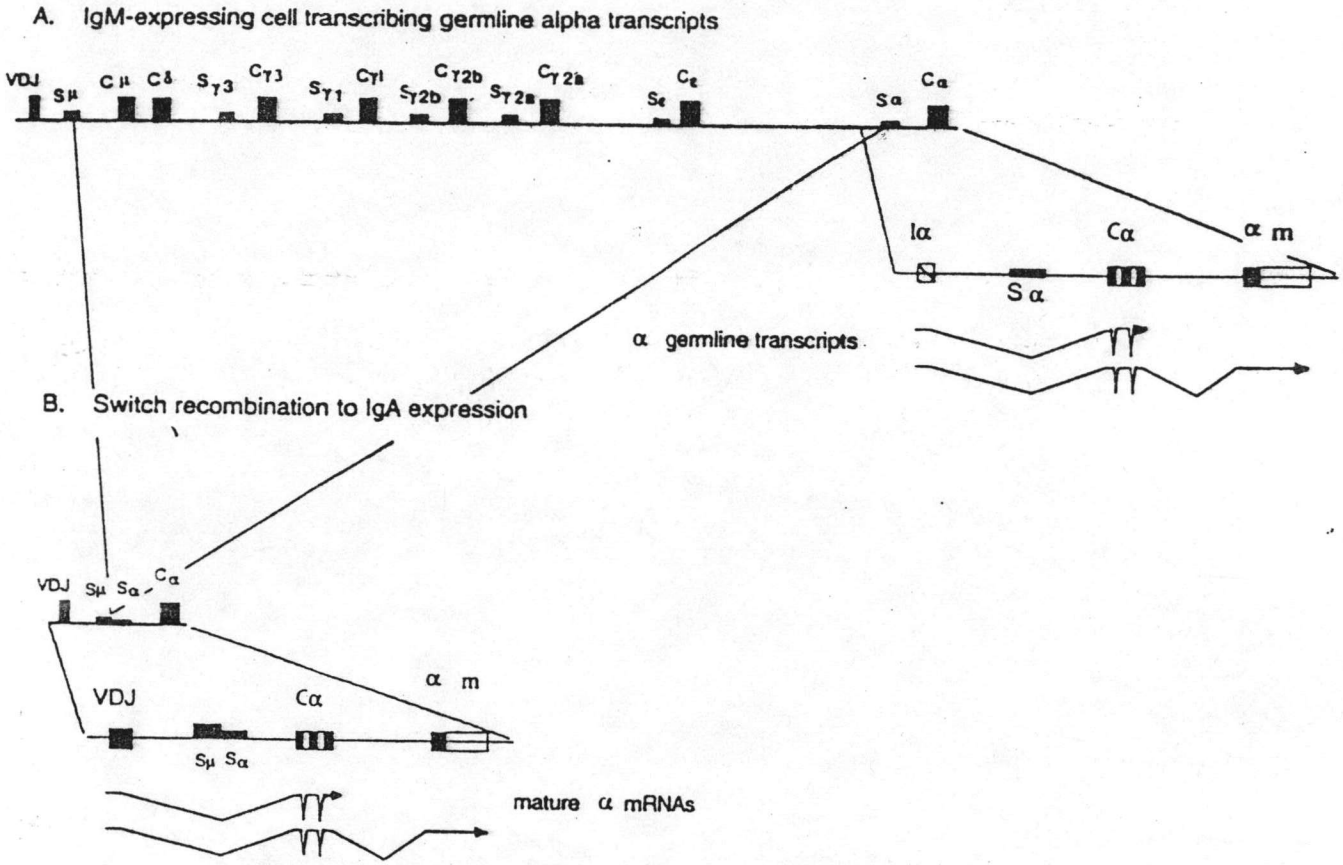

Figure 1.8 Diagram of heavy chain genes IgM-expressing mouse B cells. Map and splicing diagrams of germline α transcripts (A). Diagram of switch recombination to IgA : genomic DNA residing between the Sμ and Sα regions is excised as a circle and deleted from the chromosome (B). Shown below the expanded map of the expressed alpha gene is a splicing diagram of mature α mRNA.

1.3.7 Immunoglobulin E

Immunoglobulin E (IgE) occurs in mammals and is one of five classes of antibody recognized in man. Its role in defense against parasites was first established by work on the cell-mediated killing of schistosomes *in vitro* (Capron et al., 1982), as well as by epidemiological studies in areas of endemic schistosomiasis and other parasitic diseases (Butterworth et al., 1992 and Capron et al., 1992). IgE-secreting B cells are abundant in the skin, lungs and gut, the main sites of parasitic invasion. IgE elicits a range of cellular response to antigens, culminating in anatomical and physiological changes which exclude parasites from the body. These include inflammation, itching, coughing, lacrimation, bronchoconstriction, mucus secretion, vomiting and diarrhea, all common symptoms in allergic disorders.

Allergy is generally caused by the overproduction of IgE in response to common environmental antigens, such as those present in pollen, foods, house dust, mites, animal dander's and other natural products like rubber latex. Predisposition to allergy, however, appears to result from an interaction between genetic and environmental factors. The most common allergic diseases are asthma, allergic rhinitis (hay fever), atopic dermatitis and food allergies, but the most dangerous is anaphylactic shock, usually provoked by insect stings or parenteral medication. Allergy in one form or another affects more than 20 per cent of the population, and the alarming increase in its prevalence, morbidity and mortality over the past decade has led to its designation as the "number one environmental disease" (Wuthrich, 1989 and Barnes, 1991).

IgE and development of eosinophilia have been considered to be the most important immunological features of type I allergic reactions. Recently, evidence of a common regulation of these two aspects has appeared. In animal models the interleukins-4 and -5 (IL-4 and IL-5) have been demonstrated to induce IgE synthesis,

eosinophilia, and eosinophilic activation (Poulsen, 1995) and these properties have been confirmed in human in vitro studies. Using mouse T-helper cell subsets, designated Th1 and Th2 cells, which produced distinct cytokine profiles, most important IL-2 and interferon-gamma (IFN-) for Th1 and Th4 and Th5 for Th2. Although not all human T-cell clones will fall into one of these two IgE production (Gascan et al., 1991). Besides IL-4/IL-13, another signal -B-/T-lymphocyte interaction mediated via CD-40/CD-40-ligand-seems to be necessary for IgE production. Thus, altered responsiveness of the B cell to these stimuli could govern the degree of IgE synthesis.

1.4 Prevalence of natural rubber latex allergy

Allergy to natural rubber latex (NRL) has been acknowledged as the most important form of contact urticaria syndrome, mainly due to its connection to hazards in healthcare (Turjanmaa, 1997). NRL allergy is diagnosed by skin prick test (SPT), which has been reported as the most reliable method. The prevalence of NRL allergy is high (40-60%) in spina bifida children. The health care workers form the largest single occupational risk group (3-11%) both in Europe and in the USA. In the normal population, reports on NRL allergy was scanty, but there was evidence that prevalence was under one per cent. In general patients, one per cent prevalence of NRL allergy has been reported, where atopy and hand aczema are risk factors (Turjunmaa et al., 1996.).

1.4.1 Delayed type of latex allergy

Contact dermatitis is common in delayed hypersensitivity. The sensitizing agents are antigenic chemicals added in the valcanizing of NR household products such as sandals, gloves and elastic bands. Mercaptobenzothiazole and thiuram are known as

common sensitizers, less frequent are carbamates and *p*-phenylenediamine. In the delayed hypersensitivity, the induction and sensitization stages include development of immunized T lymphocytes able to recognize the antigen, which take about seven days. Thereafter, exposure of the sensitized person to that antigen elicits the cutaneous reaction, which peaks in approximately 48 hours.

1.4.2 Immediate type of latex allergy

Immediate latex hypersensitivity has a different immunologic mechanism, mediated by IgE. Interaction of IgE and antigen elicits massive local release of histamine and arachidonic acid metabolites, and consequently increased vascular permeability to the antigen, with systemic immediate reactions. The antigen in the IgE-mediated response is a latex protein or polypeptide.

A person with multiallergen atopic history appears to increase the risk of specific latex hypersensitivity as, of course, does frequent latex exposure (Gonzalez, 1992). Sussman et al. (1991) found that 57% of the patients sensitized to latex gloves had a background of rhinitis, bronchial asthma, eczema, or food allergy.

In Finland Turjanmaa et al. had studied immediate latex hypersensitivity and its complications occurring in sensitized physicians and nurses. They screened employees at the University Central Hospital of Tampere. All were queried about symptoms they had when wearing latex gloves and examined for hand eczema or other signs. Screening by the latex-glove scratch test revealed 23 of 512 (4.5%) employees with suspected latex-glove allergy. Skin prick test confirmed latex allergy in 15 (2.9%), all physicians or nurses. Two thirds of them had an atopic history.

The highest prevalence was observed in surgical units, gynecology and obstetrics, and Ear-Nose-Throat procedures. Over all, 7.4% of the physicians and 5.6% of nurses

in the surgical units had immediate latex allergy. Most physicians and nurses with latex-glove allergy suspected glove powder as the cause of hand symptoms and, even if they noted contact urticaria, were unaware of immediate allergy to latex gloves or the possible consequences in patients.

Latex hypersensitivity must sometimes affect patient populations in which special risk factors pertain. For example, children with spina bifida have had anaphylactic reactions with anesthesia or surgery. In the United States, Slater at George Washington University, described the problem in an eleven year old girl with spina bifida, who was admitted for a bilateral hamstring-lengthening procedure. At age of four, she had had facial urticaria and rhinorrhea after dental treatment, reactions that her family associated with the use of a rubber cofferdam. Similar reactions occurred between the age four and nine after exposure to balloons, a rubber tympanometer and a rubber face mask. Anesthesia was induced uneventfully. As the last sutures were being placed, she had a sudden increase in airway pressure. Pulse oximetry showed that oxygen saturation had dropped below 70% on 100% oxygen, and pulses were barely palpable. Epinephrine was administered intravenously and ventilation improved, but the hypotension and tachycardia persisted, requiring additional epinephrine infusion (Slater, 1989).

A second patient, also an eleven year old girl with spina bifida, was admitted for revision of a ventriculoperitoneal shunt. Her allergic history revealed no earlier reactions beyond recurrent facial and periorbital swelling when she was exposed to balloons. During the procedure, she had marked anaphylaxis with tachycardia. The reaction was reversed with epinephrine and diphenhydramine. On investigation, however, anaphylaxis was found to be due to an IgE-mediated latex hypersensitivity.

Both patients exhibited whealflare responses to 1:100 dilutions of latex and *H. brasiliensis* extracts by cutaneous injection, and to 1:1,000 dilutions by intradermal injection. The patients' basophils released histamine when incubated with the latex extracts. The sensitizing factor in plasma, presumably IgE, was significantly reduced by incubation with disks coated with anti IgE. As noted, both patients had a history of minor allergic reactions before latex anaphylaxis occurred. The routes of exposure during anesthesia and surgery might have been latex surgical gloves, breathing bags, and compression bandages, since all were used (Slater, 1989).

1.4.3 Identification of latex protein allergens

Several methods to detect natural rubber proteins have been developed and tested (Yunginger et al., 1994; Jones et al., 1994; Swanson et al., 1995; Alenius et al., 1994; Beezhold et al., 1996). The assays such as Bradford, Lowry or bicinchoninic acid protein assays have been used to estimate protein concentration in glove samples but they are not specific to the protein allergens. These assays are subjected to error because a variety of chemicals in latex products interfere in these tests (Beezhold, 1993). The Lowry method can be improved by the use of a precipitation step to remove these interfering substances (Yeang et al., 1995). Biologically, a more relevant way to quantify latex proteins is to use immunochemical methods. Several types of immunochemical assays such as Radio immunoassay, Enzyme linked immunosorbent assay and Western blot, are being employed in several countries for natural rubber protein determinations, and comparison of these methods has been reported (Beezhold et al., 1996).

In immunoblotting, latex-allergic patient sera show IgE antibodies binding to several NRL allergens, the most important of which appear to be proteins with

approximate molecular weights of 14, 20, 27, 30, 36, 45, and 75 kD (Slater and Chhabra, 1992, Alenius et al., 1993 and 1994). In the 5th International Course on Occupational Dermatology 8-13 April 1997, the current nomenclature for NLR allergens were described by Turjanmaa (personal communication) as the followings:

Heb b1 (MW 14.6 kD) = Rubber elongation factor (REF)
Hev b2 (MW 34-36 kD) = endo-1,3-β-glucosidase
Heb b3 (MW 23-27 kD) = 23-27 kD rubber particle protein
Heb b4 (MW 100-115 kD) = Microhelix protein complex
Heb b5 (MW 16 kD) = Acidic C-serum protein
Heb b6 (MW 20 kD) = Prohevein
-Heb b6.1 (MW 4.7 kD) = Hevein
-Heb B6.2 (MW 14 kD) = Prohevein C-domain
Heb b7 (MW 46 kD) = Patatin-like protein

The data clearly demonstrates that individuals react to many different latex proteins and suggests there is a large number rather than just a few major allergens.

1.5 The rational and purposes of this studies

Thailand is an important natural rubber producer in the world. The plantation areas are more than 1,900,000 ha and distributed nearly all over the country. There are more than 1.7 million people who are directly exposed to the rubber latex and about 60 million are exposed to natural rubber product as users. In the U.S. population, it was estimated that about 6.5 % may develop latex hypersensitivity (Ownby, et al. 1996).

No study has been done on the prevalence of latex allergy in Thailand. An estimation of prevalence in latex allergy was done in Finland, which was the rubber consumer, and the data was less than one per thousand (Tomazic, et al., 1992). Whilst the U.S. cases is more than 6 times that of the European.

It is difficult to obtain a study of large population of individuals who are representatives of the general population of a region. It is believed that the population that should best represent the general population of healthy adults in Thailand are samples from volunteer Red Cross blood donors. This is the reason that the prevalence of latex allergy was investigated in the blood donor group in Bangkok, where people from all parts of Thailand moved in and out regularly.

Slater, et al. (1991) and Meeropol, et al (1993) have shown that certain groups of individuals, with frequent exposure to latex, have a relatively high prevalence of latex allergy including children with spina bifida, health care workers, and rubber plant workers. Therefore, it has been planned to study the IgE of rubber allergic patients. However, in the hospital that the data was to be collected, there was no reliable routine diagnostic procedure to identify specifically for rubber allergy cases. For comparison, it is therefore assumed that the subjects of general allergy are the high serum IgE cases.

Products made from natural rubber latex include gloves, condoms and hundred of household devices. They are made in Thailand, consumed locally and also exported to

Europe and USA. Since latex allergy is associated with frequent use of high-protein allergen latex gloves and other products, efforts are underway to produce low-protein latex products. However, measuring protein allergen in latex products is problematic. Natural rubber latex is a complex and complete cytoplasm containing rubber packaged inside microscopic rubber particles. About 25 % of the latex proteins are bound to the rubber particle surface, including the enzymes required for rubber biosynthesis. Latex allergens include both soluble and particle-bound proteins (Czuppon, et al., 1994). It is believed that the bound proteins can be found in the C-serum of the latex, therefore in this study serum proteins were used as source of antigens rather than latex-associated proteins or rubber products extractable proteins.

The objectives of this thesis are :

1. to investigate the prevalence and risk of anti-latex IgE antibodies in volunteer blood donors comparing with general allergy patients.

2. to identify rubber latex protein allergens by anti-latex protein specific IgE antibodies from the donors and allergic patients.

3. to develop an immunoassay for the detection of latex protein allergens in natural rubber products.