PART - I

INTRODUCTION
CHAPTER 1. INTRODUCTION

Lectins are abundant in plant and animal kingdom. They usually are defined by their most obvious property, i.e. the ability to agglutinate red blood cells. Though the first lectins were named after the source they had been found in (e.g. ricin from Ricinus, concanavalin from Canavalia), later on the term agglutinin or phytohaemagglutinin became popular. The later name, abbreviated as PHA is now in use for the mitogenic lectins from kidney beans (Phaseolus). The term 'lectin' taken from the Latin word 'Leagre' meaning 'to choose, to select', is now most firmly established. It has been introduced by Boyd and Shapleigh in 1954. They contemplated that many of these proteins would be able to select human red blood cells according to their blood group. Later researches did not fulfil this hope, since only a very limited number of lectins proved to be blood group specific. However, the term 'lectin' has persisted.

Lectins are proteins or glycoproteins of non-immune origin that agglutinate cells and/or precipitate complex carbohydrates. The agglutination activity of these highly specific carbohydrate binding molecules is usually inhibited by a simple monosaccharide, but for some lectins di-, tri- and even polysaccharides are required.
Lectins by definition interact with oligosaccharides of cell or glycoprotein surfaces. Consequently, free oligo- or mono-saccharides of the appropriate specificity are able to inhibit or even revert this interaction. It must be kept in mind however, that lectins, though primarily acting by their carbohydrate binding sites, may reinforce their interaction, with cells or proteins by hydrophobic effect. Because of their binding specificity, lectins may be grouped together with enzymes and antibodies to form the 'affinitins' as it has been proposed by Franz and Ziska (1981).

The term 'Phytohaemagglutinin' (PHA) is generally employed for plant proteins that agglutinate red blood cells non-specifically.

Boyd and Shapleigh (1954) proposed the term 'lectins' for plant proteins behaving like phenotype whereas type specific red blood cell agglutinins of animal origin were termed 'protectin' by Prokop et al., (1968) in order to distinguish between the two. 'Protectin' involved assumption that the agglutinins may have protective immunological function.

Goldstein et al., (1980) defined lectins as 'proteins or glycoproteins of non-immune origin which agglutinate cells and/or precipitated the blood'. He suggested the term glycoconjugates.
Uhlenbruck and Krupe (1963) gave an immunochemical definition of the phytagglutinins. Phytagglutinins are extractable, globulin like substances from plant, which agglutinate red blood cells of various species. The agglutination is in many cases blood group specific.

Most lectins contain subunits which are identical or nearly identical in molecular architecture (Mr). It has been proposed to call them 'one chain lectins' (Foriers, DeNeve and Strosberg, 1979). Most of them are tetramers, typically with a Mr of about 4 x 30,000. There are some notable exceptions from this architecture, all of them occurring in the Vicieae tribe. These lectins belong to the 'two chain type' and are composed of two subunits of different size, forming tetramers of the formula $\alpha_2 \beta_2$. Another lectin of this type has been described by Kolberg, Michaelsen and Sletten (1980) from Lathyrus odoratus.

Ochoa et al., (1978) opine that most lectins possess no common feature that could be regarded as the key of their binding characteristics. Lectins are neither chemically nor structurally alike. Even those belonging to the same family or genus differ in their physico-chemical and biological properties. Ochoa et al., (1978) also proposed that the lectin specificity is a relative term and most research workers in the field when referring to a lectin inhibitor speak often about
the 'best' one, meaning thereby that there may be other inhibi-
tors with quite different structure, although less efficient. 
This problem can be of utmost importance. For example *Phaseolus
vulgaris* agglutinin is known to be inhibited by N-Acetyl-D-
Galactosamine. Curiously, the erythrocyte receptor for this
lectin lacks such carbohydrate in its structure. Further, a core
of three mannose residues was found to be relevent for binding.
These observations have been confirmed by Debray *et al.*, (1979)
and Toyoshima *et al.*, (1970). Therefore, one may also ask whether
the N-Acetyl-D-Galactosamine residues of glycoprotein are
involved in the *P. vulgaris* agglutinin glycoprotein recognition
process at all.

The terms 'specificity' and 'reactivity' of the
phytagglutinins or lectins require clarification. Specificity
and agglutinin content are definitely not constant and probably
depend on some factors, such as geographical location of the
vegetation, maturity of the seeds, freshness and oldness of the
seeds, concentration of the phytagglutinin solution, the partic-
cular portion of the plant used (leaves, fruits, seeds or bulbs,
etc.) (Tobiska, 1959; Makela, 1956; Reimann, 1960; and others).
Known agglutinin carrying species may prove to be agglutinin
free or their degree of agglutinating reactivity may be decreased
in the same species from another geographical location.

Regarding concentration we can say that certain lectins
have their own optimum concentration to react with the red blood cells e.g. *Dolichos biflorus* cannot react optimally when in high concentration.

Extracts of freshly gathered seed of *Bandeiraea simplicifolia* agglutinates both A and B cells in the sequence, strongest in B cells, stronger in A,B and strong in $A_2B$ (Makela, 1957).

Reimann (1960) reported that *Sophora japonica* seed of Bulgarian origin mainly contains a B-agglutinating substance, while the pod contains mainly an anti-A. In contrast to the 'B-type' of the seed, the 'A-type' of the pod reacts with 0 cells of man but not with any other animal blood except that of rabbit and pig.

There are certain genetic determination of the occurrence of specific phytagglutinins in a plant; even between two plants of a same species with different genetic endowment, marked difference regarding reactivity has been reported. Renkonen (1948) investigated samples of the plant *Vicia cracca* (normally the anti-A carrying plant) from various localities and found that those species whose chromosome number was 28 or 14 were active but others with 12 or 24 chromosomes were inactive. Ottensooser (1955) confirmed this phenomenon.

Recently, some authors also opine that there is also a
genetic regulation on the susceptibility of the red blood cells, or their reactivity against certain lectins. Different samples of same blood groups may show differences in degree of reaction (as weak, medium or strong) against certain specific lectin.

According to some 'lectinologists', the term 'lectin' should be used to designate only the blood group specific plant agglutinins, while the term 'phytagglutinin' should be used to designate the non-specific plant agglutinins and the term 'phytohaemagglutinin' (PHA) should be used to designate those plant agglutinins which have the mitogenic properties. However, we commonly use the name 'lectin' as an umbrella term for all cell agglutinating or glycoconjugate-precipitating proteins or glycoproteins of non-immune origin, irrespective of their source, plants or animals.
BRIEF HISTORICAL ACCOUNT

In the present context, although Stillmark first discovered a lectin in 1888, it was not until 1916, that the haemagglutinating property was exploited by Dorset and Henly using an extract of red kidney bean (*Phaseolus vulgaris*), a technique which subsequently became popular for separation of leucocytes and nucleated erythrocytes from blood and bone marrow. This led to the introduction of a new technique in 1955 by Osgood and Krippahne for culturing peripheral blood lymphocytes, aimed at studying the developmental relationship of white blood cells. It was in the course of such a study that Nowell (1960) observed the development of blast cells with primitive features in cultures of normal blood leucocytes. The mitogenic effects of phytohaemagglutinins on lymphocytes was soon confirmed and established by later studies.

It has been known for about a century that certain plants contain substances that are capable of agglutinating erythrocytes and other cells. Such agglutinins are found predominantly in the seeds of plants, in particular those of the leguminous plants. They are present also in other plant tissues such as roots, leaves and bark (Krupe, 1956; Makela 1957; Bird, 1959; Boyd, 1963; Toms and Western, 1971). There is evidence for their presence in microorganisms, invertebrates (Gold and Balding, 1975) and vertebrates (Stokert *et al.*, 1974; Teichberg *et al.*, 1975).
The ability of an agglutinin to distinguish between different types of human cells provided the basis for the term 'lectin' which is derived from the Latin word 'Leagre' meaning to select or to choose.

The early studies of haemagglutination plant extracts involved only a few species e.g. *Ricinus communis*, *Abrus precatorius*, *Croton tiglium* and *Robinia pseudoacacia*. The lectins obtained from these plants were termed recin, abrin, cortin and robin respectively after the name of the plants.

Lectins also exhibited toxicity and due to this property they were collectively referred to as 'Phytotoxins' and their toxicity was mistakenly attributed to their haemagglutinating ability. Muller (1899) was perhaps the first to show that the toxic and haemagglutinating components were separable.

Landsteiner and Raubitschek (1907) found agglutinins for the first time in the seeds of *Phaseolus vulgaris*, *Pisum sativum*, *Lens esculenta* and species of *Vicia*. All these belonged to the family *Leguminosae*, subfamily *Papilionaceae*. Later, Wienhaus (1909) coined the term 'phasins' for these non-toxic, haemagglutinating plant extracts. These workers also found agglutinins for the first time in the milky juice of Spurge plants (*Euphorbiaceae*). This discovery along with that of Korbert (1906) showed the presence of agglutinins in material other than seeds.
Expressions like 'glaibrification' and 'conglutination' were used to describe the activities of the 'phasins' until Effstrand (1898) introduced the terms 'haemagglutinating' and 'haemagglutination' to the phenomenon of the clumping of red blood cells, a finding supported by Landois (1974) also.

The experiments of Ehrlich (1891) broadened the scope of lectins by immunizing mice with increasing doses of 'ricin' and 'abrin'. He found a vastly increased tolerance in mice against 'ricin' as compared to unimmunized control mice. The antigenicity of lectins together with their haemagglutinating activity and the fact that the lectins were easier to prepare and tested in the laboratory provided grounds for their wide acceptance in immunological research in later years.

Renkonen (1948) found 6 kinds of group specific agglutinins in addition to 31 non-specific and 62 ineffective ones amongst the seeds of 99 species of Papilionaceae. The historically interesting lectins found by Renkonen (1948) were obtained from various species of Vicia cracca, which contained anti-A and a smaller amount of anti-B substance.

2. From *Lotus tetragonolobus* containing substances directed against O and A₂ blood and suspected of being anti-H.

3. From *Laburnum alpinum* and *Cytisus sessilifolius*
possessing anti-O(H) lectin.

Boyd and Reguera (1949) investigated 262 plant species belonging to 63 families and were successful with 70 different kinds of American beans, specially the Lima or lymar bean, Phaseolus lunatus (syn. Limensis) which showed strong and specific anti-A activity. These studies inspired many other workers like Cazal (1952) and Lalaurie (1952), Ottenssooser (1955), Bird (1956), Makela (1957) and Tobiska (1959), etc., to look for serological properties of lectins in other plants. Not only seeds, but leaves, bark, roots, bulbs, etc. were also investigated, so much so that lower plants like Fungi were also screened. The total number of plant species examined was estimated to be about 1000 by Ottenssooser in 1955 which increased to about 3000 by 1959 (Tobiska, 1959). The aim of the search for phytogglutinins was mainly to find group specific vegetable reagents for routine tests, and in this way, many plants with haemagglutinins were discovered.

According to an estimate by Tobiska (1959), about 222 specific vegetable reagents for A,B,H and N were known till 1960, most of these being anti-A extracts. Bird, Ottenssooser and Reimann (1960) occupied themselves with the particular question of the 'species-specificity' of phytogglutinins which proved to be extremely important in the forensic field.

The work on blood group specific lectins took a leap
after 1950 when some important discoveries were made. Bird (1951) reported strong anti-A activity in the seeds of Dolichos biflorus (the most common edible pulse crop in northern and western India). Cazal and Lalaurie (1952) stated the presence of strong anti-H activity in the seed extracts of three species of Ulex. An agglutinin behaving like anti-N was obtained from Vicia graminea by Ottenssooser and Silberschmidt in 1953. In 1959, the anti-'GY' lectin was obtained from the peanut (Arachis hypogea) by Boyd et al., (1959). In 1959, Yugoslav workers Mitrovic and Simonovic in a short publication, pointed out that the extraction of anti-Rh (anti-D) from plants like Begonia punctata might be possible after immunization. A lectin which behaved like anti-H I or I/HI was obtained from the seeds of Sophora japonica plant (Chien et al., 1974).

Shrivastava et al., (1979) described a new red blood cell membrane specificity which was detectable by seed extracts of Erythrina lithosperma. This new specificity has been designated 'LH' after the name of Polish serologist Ludwik Hirszfeld (1971) and has not been found to be identical with any, so far known, red blood cell specificity.

Now-a-days, the lectins are indispensable haemagglutinin and occupy an established place in routine serological work. Aqueous solutions in saline are nearly always used, because these not only are satisfactory but have proved themselves
superior to alcoholic extracts. The number of lectins having serological properties is now legion and it is perhaps neither feasible nor desirable to catalogue them all. Listing all phytagglutinins would require a separate monograph (Krupe, 1956; Makela 1966 and Tobiska 1959). It is for this reason that Potapov (1968) introduced an expression 'lectinology' for this vast field.

**Blood Group Reactive Lectins**

There are several blood group specific lectins discovered so far which identify several antigens on the erythrocyte membrane. Besides plants, they may be obtained also from certain bacteria (as *Escherichia coli*), Fungi (as *F. fomentarius*), fish, Molluscs, Gastropods, etc. (Bog-Hansen, 1980). However, not all lectins are found to be suitable laboratory reagents for considerations such as difficulty in obtaining raw material, instability under normal storage conditions, dependable on proteolytic enzymes for optimal reactivity, low concentration of agglutinins in the seeds and failure to react with weakly expressed antigens.

**Lectins Identifying 'A' Red Blood Cell Antigen (Anti-A Lectins)**

Anti-A was the first phytagglutinin with blood group specificity to disclose itself. Renkonen (1948) showed that it could be of practical use in making the A_1-A_2 distinction.
Boyd and Reguera (1949) found it in *Phaseolus limensis* (Lima Bean). Now-a-days, *Vicia cracca* is not used generally because this anti-A is not pure, and in addition contains anti-B and anti-H.

For the typing of blood group A and the subgroups of it, as \( A_1, A_2 \) etc. (also in the differentiation of \( A_1B \) and \( A_2B \) groups), seed extract of the *Dolichos biflorus* is used universally because of its efficiency, easy availability and simple preparatory method. Simple physiological saline (0.85%) extracts yield high titre and specific anti-\( A_1 \) reagents. Bird in 1951 discovered it and opine that its efficiency against \( A_1 \) is about 500 times stronger than against \( A_2 \). Interestingly, whereas the concentrated extract fails to subtype blood group A, it becomes specific anti-\( A_1 \) reagent upon suitable dilution.

Boyd and Shapleigh (1954) found that the *Dolichos biflorus* extract would precipitate with the saliva of secretors of group \( A_1 \) but not \( A_2 \). Bird (1959) found that his purified extract would precipitate with both \( A_1 \) and \( A_2 \) secretor saliva, although the precipitation being heavier with \( A_1 \) saliva. Bird (1959) concluded that this lighter precipitation with \( A_2 \) saliva than with that of \( A_1 \) saliva was only a quantitative difference and not a qualitative difference. Other advantages of the use of *Dolichos* anti-A lectin include:

1. that it does not react with O or B cells suspended
in albumen or treated with enzymes as other seed
anti-A preparations tend to do,

(ii) the extract can be purified as a very avid anti-A₁
by absorption technique, and

(iii) that it does not react with the 'Foresman antigen'
(Bird, 1952) nor with the T-antigen of 'changed cells'
(Bird, 1954).

Anti-A lectins are highest in number among all the blood
group specific lectins discovered so far. It has been found in
only one non-leguminous plant, Hyphtis suaveolens of the order
 Labiatae (Bird, 1959, 1960). Some animals also yield anti-A
lectin e.g. Helix pomatia, H. hortensis, Euphadra periomphale
(all snails); Tapes literata (a shell fish), etc.

Lectins Identifying B Red Blood Cell Antigen (anti-B lectins)

As far as known to the author, there is as yet no such
convenient lectin reagent for the typing of group B red blood
cells, notwithstanding the fact that anti-B activity has been
reported in many plants and animals. Reliably pure anti-B
extracts of vegetable material have not been made available up
till now. All exhibit mixed specificity. It appears that most
anti-B reagents from these sources are quite often unstable and
incomplete.

Elo, Estola and Malmatrom (1951) found a substance
similar to anti-B in the extract of mushroom, *Marasmius oreades*.

The anti-B from *Vitis aestivalis* (Boyd and Reguera, 1949) is weak. Krupe (1956) described *Sophora japonica* and *Coronilla varia* originally as anti-B but later it was recognized as anti-(B+A). Concealed B-affinity was found by Reimann (1960) in preparations from potatoes; inhibition with peptone left only B-effect.

Makela and Makela (1956) prepared one anti-B like extract from 'old' samples (at least one year old) of the seeds of *Bandeiraea simplicifolia*.

A moderately strong anti-B was obtained by extraction from the fungus (Makela et al., 1959).

Rogers et al., (1977) isolated anti-B from the red marine algae, *Ptilota plumosa*.

Extracts which agglutinate A and B cells more strongly than O cells have been prepared from the following seeds - *Sophora japonica* (Krupe et al., 1956); *Bandeiraea simplicifolia* (fresh seeds) (Makela et al., 1956); *Crotolaria striata* (Bird, 1956); *Calpurnia aurea* (Bird, 1957); and *Crotolaria mucronata* (Ottensenoser and Sato, 1963), etc.

The general conclusions seem to be that the anti-A and
the anti-B are one agglutinin reacting with some anti-genic component, which is common to both A and B cells, and thus closely resembling the cross reacting antibody in group O serum.

Morgan et al., (1953) and Furuhata et al., (1959) demonstrated some important facts in this field. The Sophora japonica lectin showed a stronger affinity for B than for A cells and Coronilla varia also showed the same reactivity. Both phytogglutinins are specific for O cells (Krupe et al., 1954).

Reimann (1960) reported that Sophora japonica seed (of Bulgarian origin) mainly contained a B-agglutinating principle, while the pod contained mainly an anti-A. In contrast to the B-type of the seeds, the 'A type' of the pod also reacts with O, but not with animal blood ('B-type' positive with rabbit and pig).

After prolonged storage, seeds of the B. simplicifolia produced extracts which reacted only with cells containing (B, A₁B, A₂B) (Makela et al., 1957). Gold and Balding (1975) however, were unable to confirm anti-B specificity in the stored seeds of this plant species and Murphy (1989) found only anti-A/B specificity in both stored and fresh seeds.

this lectin cannot be used for blood typing as it is not possible
to separate the anti-A and anti-B ingredients. Two other lectins
have also been isolated from these seeds in addition to the one
described by Hayes and Goldstein (1977). One of these has been
called BS-I. Iyer et al., (1976) isolated an N-acetyl-D-glucos-
saminyl binding lectin and designated it BS-II. This lectin has
been shown to possess anti-TK specificity (Judd et al., 1977). A
third lectin, BS-III appears to have no blood group specificity
(Murphy 1980), Murphy and Goldstein (1977) found that BS-I com-
prised of five isolecitins viz. BS-I, BS-I(Au), BS-I(A3B), BS-I
by mixing BS-I isolecitin to small quantity of N-Acetyl-D-
galactosamine.

The extracts of other plant seeds showed a stronger
action against A, but their specificity against O (anti-H)
remained unchanged, e.g. Crotalaria striata (Bird, 1956),
Crotalaria compista (Makela, 1956) and Caragana frutex var.
Latifolia (Makela, 1957). He made the interesting observation
that the extract of Caragana frutex reacted only against red
blood cells in a serum medium and not when they were in saline
suspension.

According to Ottensooser et al., (1958) the extract of
Crotalaria mucronata and of a variant of Phaseolus lunatus showed
reaction of different intensity against different B blood samples
in identical conditions but the intensity of each reaction
remained almost constant. The reactions were a little stronger with the red blood cells from adults.

These anti-(A+B) lectins have been classified as 'A stressed', 'B-stressed' and 'A=B' according to their reactivity.

There are some preparations which contain both anti-H and anti-B, which may be separated. These extracts are not obtained from Leguminoseae, but from Celestraceae. The most important species is the spindle tree, Evonymus europaeus (Schmidt, 1954). The carriers of the agglutinating principle are the orange red membrane of the ripe seed (Krupe, 1954) while the seed itself contains practically no agglutinin (Schmidt, 1954). Tobiska (1959) reported that Evonymus planipes, extracts show specific, but relatively weak anti-B effect. On the contrary, the extract of E. vedonensis seemed to contain anti-B and anti-H (+anti A7). Prokope et al., (1969) showed that its activity was enhanced by pronase treatment of both human and animals cells. Ottensooser et al., (1972) reported that the formation of anti-B specificity by variety of Evonymus sp. was due to Fusarium roseum living in it.

There are some animal anti-B lectin also, reported from spawn (Potapov, 1974), snail, etc.

Rose (1982) used peanut lectin from Arachis hypogaea to subdivide lymphocyte populations.
Nasholt and Sorensen (1982) succeeded in using Con A as a vehicle for the introduction and retention of one of the two galactosidases into cultured fibroblasts in Fabry's disease and \A-linked hereditary disorder due to deficiency in the synthesis of these enzymes.

Sellaror et al., (1982) demonstrated the presence of a lectin by fluorescent microscopy around colonies of a blue green algal symbiont, \textit{Anabaena azollae}.

Bazil et al., (1983) studied on components of lectin obtained from the ooc. tes of \textit{ Aerco. Verco. fluvialitris} by adsorption technique using D-glucose.

Baldstrom et al., (1983) studied on the role of lectins in respiratory infections and in organotrophy due to some diseases.

Krajhanzl and Pocourack (1986) discovered bactericidal static effect of the reach cortical lectin.


**Lectin with Anti-H Reactivity**

Besides the anti-A effective lectins, these form the largest group of the identified specific lectins. They react both with O cells and with A2-cells more strongly than with A1.
The most important ones are given in table below.

**Plant Seed and Preparations Showing Anti-H Activity**

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Laburnum alpinum</em></td>
<td>Renkonen (1948)</td>
</tr>
<tr>
<td>2.</td>
<td><em>Lotus tetragonolobus</em></td>
<td>Renkonen (1948)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Cytisus argenteus</em></td>
<td>Cazal and Lalaurie (1952)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Cytisus sessilifolius</em></td>
<td>Renkonen (1948), Krupe (1953)</td>
</tr>
<tr>
<td>7.</td>
<td><em>Cytisus purpureus</em></td>
<td>Renkonen (1948)</td>
</tr>
<tr>
<td>8.</td>
<td><em>Ulex europaeus</em></td>
<td>Cazal and Lalaurie (1952)</td>
</tr>
<tr>
<td>9.</td>
<td><em>Ulex gallil</em></td>
<td>Cazal and Lalaurie (1952)</td>
</tr>
</tbody>
</table>

The various anti-H preparations can be used in practice, not only for the testing of red blood cells—the most important being the determination of sub-group $A_2$ comparable with $A_1$-extracts: Kouzumies (1949) but also for the establishment of the secretor status (Boyd and Shapleigh, 1954) and for the direct proof of 0 in spots of blood, saliva and seminal fluid. Secretor saliva of all groups inhibits vegetable anti-H due to secretion of H-substances which is common to all secretors. The practically
H-free group A1B is however, unsuitable for this purpose (Race and Sanger, 1950).

Among the anti-H reagents discovered so far, *Ulex europaeus* is most commonly used for its efficiency. It usually agglutinates red blood cells in the following order of strength:

\[ 0 > A_2 > A_2B > B > A_1 > A_1B \]

Voak and Lodge (1971) are of the opinion that anti-H lectin from *Lotus tetragonolobus* in fact, may be anti-H1 since it does not agglutinate adult O1 negative cells and O1n cells (the Bombay phenotype). Likewise, the anti-H from *Cytisus sessilifolius* is considered to be anti-H/H1 since it reacts less readily with O1 negative cells than with O1 positive cells unless pretreated with a protease or neuraminidase. These experiments led Bird (1976) to conclude that the observed differences may be due to steric inter relationship between H1 and sites rather than variation in the anti-H reacting specificity. The lectin of *C. sessilifolius* is purified according to the method described by Matsumoto and Osawa (1974).

Extracts of the mushrooms, *Pleurotus ostreatus* and *P. spodoleucus* contain an anti-H specificity (Kogure, 1975).

Prodanov and Atanasova (1980) extracted an anti-H from leaves of *Sambucus nigra*. The discovery of anti-H lectin in
the seeds of *Ulex europaeus* by Boyd and Shapleigh in 1954 made the establishment of secretor/non-secretor status easy. Group A, B and AB people had for long been easily divided into secretor or non-secretors, but it was difficult to classify people of group O, due to lack of anti-\(H\) reagent; human with good anti-\(H\) in their serum were too rare, eels too difficult to handle and *L. tetragonolobus* too awkward to pronounce. It was the use of *L. tetragonolobus* that led Bird (1954) to give his theory about the relationship of anti-\(Le^b\) to anti-\(H\) (in his opinion, \(Le^b\) is a partial antigen of \(H\), perhaps \(H_1\) and anti-\(H\) may be thought of as anti (\(H+H\)).

**Lectins Identifying M and N Blood Groups**

Allen and Brilliantine (1969) discovered anti-M activity in three species of *Iberis*. Soon thereafter, Okada and Nakajima (1970) found similar activity in some varieties of Japanese radish and turnip. Romanowska (1964) worked with *Iberis amara* and observed weak anti-M activity in its ammonium sulphate precipitated extracts. Hak (1972) reported anti-M activity in *Raphanus sativus* and Moulds and Judd (1977) demonstrated this activity in *Iberis umbellata* seed extracted either in glycine or bovine albumin. But this reagent was not found suitable for red blood cell typing as its activity is influenced by the Ss-antigen status of cells. A fairly good anti-N reagent was prepared from the seeds of *Vicia graminea* (Ottensooser and Silberschmidt, 1953 and Potapov, 1974). This lectin has now been purified by
Prigent and Bournillon (1976). An anti-A/N activity has also been reported in one of the isolectins obtained from

Lectins Identifying P Blood Groups

Although no lectin specific for P blood group has perhaps been reported, there do exist lectins which react preferentially with blood group P and B. Anti-B/P activity has been reported in Salmonidae roe by Voak et al. (1974), while Kuznetsova (1976) found an anti-B+P activity from cavier of Rutilus rutilus. Anstee et al. (1973) however could not confirm presence of anti-P₁ in R. rutilus extract. Schnitzler et al. (1968) found that the anti-B and anti-P₁ activities of extracts from the roe of R. rutilus were inseparable.

Lectins Reacting with I Red Blood Cell Antigen

Lectins reacting specifically with the I antigen are not known but there are some which behave like anti-HI or anti-I/Hi. Experiments with Sophora japonica lectin (Chien et al., 1974) showed that it agglutinates cord cells and adult AI negative and BI negative red blood cells less readily than it does AI positive and BI positive red blood cells. This lectin did not agglutinate
group O red blood cells and therefore, has been considered anti-AI/BI. An anti-B lectins from the ova of *Spondylosoma cantharus* was found to react preferentially with adult BI positive red blood cell (Rogers, 1978).

**Lectins Reacting with Other Blood Group Antigens**

**The Gy system:** Boyd *et al.* (1959) working with *Arachis hypogea* lectin described a new blood group factor called Gy by them. Another lectin from the same plant was found to possess anti-T activity (Bird, 1964). Whether these two activities reside in the same lectin or there are two distinct lectins with these specificities is not yet clear.

**The CL system:** By absorbing extracts of seeds of the Korean *Clerodendron trichotomum* with selected human group O red blood cells, Wiener and Moon (1975) described a new specificity designated as CL system. This specificity appears to characterise a structure associated with A-B-H-Le macromolecule, both of red blood cells and of saliva, which is distinct from the combining groups for A,B,H and Le. The reactivity of red blood cells with anti-CL lectin gets destroyed by treatment with proteolytic enzymes, unlike the reactions for A,B and O.

**Lectins as Polyagglutinins and Panagglutinins**

Polyagglutination described the conditions where red blood cells were agglutinated by high proportion of ABO blood group
compatible with normal adult human sera (Judd, 1980). The term polyagglutination and panaagglutination are quite often used synonymously, although Bird (1971) prefers to treat them separately. According to him, panagglutination is a non-specific agglutination of normal erythrocytes due to a number of substances present in serum, whereas polyagglutination is agglutination by a variable number of sera of erythrocytes which may be abnormal in one way or the other.

Lectins are widely used in the recognition and classification of polyagglutination. There are at least nine forms of lectins known today for this purpose.

**T-Activation:** T-activation of erythrocytes is dependent on the activity of neuraminidase which exposes latent T receptors by cleaving N-acetyl neuraminic acid from red blood cells. Bird (1964) reported an anti-T lectin obtained from *Arachis hypogaea*. Issitt et al., (1972) found that the T-receptors could be destroyed by ricin but not by papin. Gunson et al., (1970) observed destruction of T receptors by both these enzymes. Bird et al. (1978) observed that the T-activated red blood cells reacted also with Glycine max lectin apparently because of the reduced NANA content in it. Donald (1979) described method for preparing anti-T lectin, anti-T absorbed serum and purified anti-T antibody.
Th-Activation: Bird et al., (1978) described this type of polyagglutination which is also induced by bacterial enzymes. Since the NANA content is normal in the Th-activated cells, they do not react with Glycine max.

Tk-Activation: Erythrina indica and Sambucus nigra lectins may be used to distinguish between T and TK activation. The TK activation as a distinct entity, was first recognised by Bird and Wingham (1972). An anti-Tk activity was reported also in lectin (BS II) from B. simplicifolia (Judd, 1977).

Tn-Polyagglutination: Bird and Wingham (1974, 1976a) have reported anti-Tn or anti-Tn\(^+\) anti Cad reactivity in saline extracts of seeds of several species of saliva. The more commonly used of such lectins are S. sclarea and S. hortinum.

The Tn Polyagglutination was first described by Moreau et al., (1971). Unlike the polyagglutinations mentioned earlier, this condition does not arise by direct action of bacterial enzyme. Instead, it appears to be caused by some defect in the red blood cell membrane. It is interesting to note in this context that Tn-polyagglutinable red blood cells lock a red cell membrane Galactosyl transferase (Cartron, 1978).

Acquired B Polyagglutination

This type of polyagglutination can be acquired by the action of bacterial enzyme, e.g. certain strains of Escherichia coli.
(Springer et al., 1961). The action altered the red cell membrane in such a manner that it produced a B-like receptor. This reaction is perhaps caused by a bacterial enzyme deacetylase which converts N-acetyl-D-galactosamine to galactose (Gerbal et al., 1970 and 1975). The acquired B polyagglutination may be studied with the help of Dolichos biflorus lectin.

**Cad Polyagglutination: Dolichos biflorus** lectin has found use also in studying Cad polyagglutination. This type of polyagglutination was first described by Cazal et al., (1968). So far, three phenotypes in this system have been recognised. Only one of these, strong Cad positive, is polyagglutinable. Cad positive red blood cells were agglutinated also by S. hortinun and S. forinace seed lectins (Bird and Wingham, 1976a). Their agglutination by Glycine max lectin is believed to be due to the presence of exposed D-gal-NAC residues (Bird and Wingham, 1974) and not due to any reduction in NANA content (Cazal et al., 1968).

**Hereditary Erythroblastic Multinucularity with Positive Acidified Serum Test (HEMPAS)**

This is an inherited anemia associated with structural abnormality of red blood cell membrane (Crookston et al., 1969, 1972; and Vervilghen et al., 1973). The H antigen in HEMPAS erythrocytes has been shown to be depressed with anti-H agglutinins from Cytisus sessilifolius seed and from the eel,
**Anguilla anguilla.** Further *Haliotis aurantiaca* and *Wintaris sinensis* have been found to react more strongly with HEMPAS than with control cells and an agglutinin from albumin gland of *Helix pomatia* agglutinates weakly group O HEMPAS cells but not control cells (Bird and Wingham, 1976b).

**NOR and VA Polyagglutination**

The VA (Vienna) polyagglutinable red blood cells react weakly with anti-H lectins and may be taken as an example of bacterial enzyme induced form of polyagglutination (Graninger et al., 1977). The NOR polyagglutination probably is an inherited condition (Harris, 1979). Since the lectins identifying polyagglutinable red blood cells are not known, the information about NOR polyagglutination is scanty.
FUNCTIONS OF LECTINS

There seems to be no general consensus regarding the role of lectin in nature. Bohlool (1974) attributed nitrogen fixing property of leguminous plants with their lectins, a view also shared by Wolpert and Albersheim (1976). It has been suggested that lectins offer protection by interacting specifically with viral and fungal polysaccharides (Bird, 1959). Prokop et al., (1968) and Fountain et al., (1977) found that lectins played a role in sugar transport but there does not seem to be much evidence to support this hypothesis. In view of mitogenic properties, it is possible that they control cell division and germination in plants (Sharon and Lis, 1972).

Recent investigations have shown that animals also contain lectins, e.g. in the liver-cell membrane. These lectins bind to galactose residues which often constitute the subterminal sugar of serum glycoproteins. Aged glycoproteins i.e. those which have lost their terminal sialic acid residues, are believed to be recognised by the lectins and thus get removed from circulation.

The three international meetings on lectin held at Copenhagen on May 23, 1978, on April 3, 1979 and on June 8, 1980, have provided precious informations about lectins, their biology,
and biochemistry etc. However, much more remains to be investigated. The subject has aroused so much interest in Haematologists that the following aspects require further research work:

(i) What is the biological function of lectins? Do they have an essential function with respect to the life of the plant when even some members of the same family lack them?

(ii) Can lectins have a function within the plant, specially when sugars (with which they react) do not occur in plants at all?

(iii) What is their evolutionary relationship in different plant tribes?

(iv) Is there any relationship in amino-acid sequence and lectins in plant life?

As regards the biological function of lectins, the argument is indirect, but nevertheless valid. Leguminosae seeds contain considerable amounts of lectins. In most cases e.g. pea, broad bean, and lentil, etc. about 0.1% of the seed weight is lectin. In jack beans, the lectin content reaches upto 3% of the total weight. It cannot be imagined that nature will accumulate a particular protein in such big amounts without necessity. Extension researches are needed to formulate the biological function of lectins.
Gansera et al., (1979) and Gebauer et al., (1979) reported the presence of lectin binding proteins in plants. There always exists at least one protein which is specifically recognized by the lectin from among the many proteins of the plant (Rudiger et al., 1981). This fact indicates that lectins have a definite role in the physiology of plants.

The third argument is concerned with the lectin amino-acid sequences. Lectins of different tribes of Leguminosaeae are quite homologous (Foriers et al., 1979 and Baumann et al., 1979). A compilation of some of the Viciae lectin sequence was presented by Baumann et al., in 1981. This high degree of homology in lectin sequences reflects an important though unknown biological function.

Also the 'medical future' of lectins has interesting aspects. Possibly most promising is the action of toxins in hybrids, synthesized of lectin and toxin as 'therapeutical' tumour markers (Uhlenbruck et al., 1980).

Role of Lectins in Nature

The role of lectins in nature, whether in plants or in animals remains a mystery. Bird (1959) and Boyd (1963) suggested that they are antibodies intended to counteract soil bacteria. Alberscheim and Anderson (1971) proposed that they serve to protect plants against fungal attack. Sharon and Lis (1972)
postulated that because of their saccharide binding capacity they are involved in sugar storage and transport. Keeping in view their mitogenic activity, they suggested that lectins might be involved in the control of cell division and germination in plants or they might act as glue for the attachment of glycoprotein enzymes in organised multienzyme systems.

Hamblin and Kent (1973) and Bohlool and Schmidt (1974), proposed that lectins may serve to bind nitrogen fixing bacteria to the root nodules of leguminous plants. Lectins may help to protect plants against certain phytopathogens (Mirelman et al., 1975) and insect predators (Janzen et al., 1976). On the other hand, Kauss and Bowless (1976) suggested that lectins may be involved in the regulation of plant cell wall extension.

Ofek et al., (1977) found lectin like substances on the surface of bacteria and hence postulated that they might be responsible for their adherence to and colonization of human epithelial mucosal cells.

There is hardly any conclusive evidence for these proposed roles by various workers. Since many plants which are apparently devoid of lectin do flourish, it is possible that the biological properties so far encountered have no relation to their function in nature.
HISTORICAL REVIEW OF WORK DONE IN INDIA

Shrivastava et al. (1979) were perhaps first to work on lectins in India at Patiala. These workers described a new red blood cell membrane specificity which was detectable by seed extracts of Erythrina lithosperma. This new specificity, termed 'LH', was not found to be identical with any known red blood cell specificity.

Sehajpal and Shrivastava (1980, 1981) continued their researches on the newly discovered LH specificity and showed its distribution in some Indian populations.

Reddy et al. (1981) working independently at Calcutta with LH specificity and its immuno-chemical properties agreed with the findings of Sehajpal and Shrivastava (1980).

Roy and Bhalla (1981) at Chandigarh worked with haemagglutinins and lysins of plant origin and studied their application in characterising human and animal red blood cells.

Ghosh et al. (1981a, 1981b) isolated and purified lectin from seeds of Butea monosperma and Momordica dioica by affinity chromatography with bacto-sugar and found that these agglutinated erythrocytes of higher vertebrates like man, rabbit, chicken,
and also protoplast solutions of some plants.

Bhattacharya et al., (1981) studied the immuno-chemical properties of lectins obtained from four species of *Erythrina*.

Sandhu and Reen (1982) and Sandhu et al., (1982) studied distribution, specificity and in vivo function of phytolectins, haemagglutinins or haemolysins.

Sehajpal (1982) described CL system from the leaf extract of *Clerodendron inermae* and demonstrated that it reacted with human erythrocytes in three different ways and classified them into three types, $CL_s$, $CL_w$ and $CL_{negative}$. Tewari (1985) showed that these three CL types were under autosomal genetic control.

Kaur (1983) worked on the LH red blood cell membrane specificity in man and selected animals.

Pelia and Sandhu (1988) investigated into lectins in 400 Indian plants using human and animal erythrocytes, lymphocytes and spermatozoa.
OBJECTIVES OF PRESENT STUDY

It is evident from the description given above that the study of lectins is very interesting and promises to be of great advantage in serology as well as in understanding many biochemical processes in cells. It is also clear from the scanty literature available that not much work has been done in India and it is only recently that the scientists have begun to take interest in lectins. The author, therefore, undertook the present study under an inter-disciplinary research collaboration on the suggestion of Dr. P.K. Shrivastava, Professor of Anthropology and Head of the Department, Doctor Harisingh Gour Vishwavidyalaya, Sagar mainly in view of the enormous variety of flora and fauna available in this country and usefulness of lectins in serology. The work on "Studies on Lectin-Cell Interaction" was started in January, 1988 under the joint supervision of Dr. B.K. Srivastava, Professor of Zoology and Head of the Department and Dr. P.K. Shrivastava, Professor of Anthropology and Head of the Department, Doctor Harisingh Gour Vishwavidyalaya, Sagar chiefly with the following objectives:

1. to make a survey of Indian flora and extract & identify new lectins from Indian plants,

ii. to identify new lectins of serological interest,
iii. to differentiate among red blood cells of various animals by using lectins,

iv. to differentiate between human blood groups using specific lectins, and

v. to gain more knowledge about lectin biology.

The present thesis embodies the work done on the aforesaid lines and the findings of study are incorporated in the subsequent pages. For the sake of convenience of description, the thesis has broadly been divided into four parts, Part I - Introduction, Part II - Materials and Methods, Part III - Experiments and Results, and Part IV - Physico-Chemical studies, followed by Bibliography.