Environmental agents such as pollen grains have been known to initiate the allergic response in the susceptible host for over a century and a half (Stanley and Linskens, 1974). This allergy was found to be an adverse physiological consequence of the interaction of allergens (i.e. antigens) with humoral antibodies. The body's immune system regulates the response of specific skin sensitizing and reaginic antibodies, to foreign allergens. Allergens are proteins or glycoproteins that are capable of eliciting this immune response in the susceptible host (Ishizaka and Ishizaka, 1973; Pepys, 1973; Kaltreider, 1976). The IgG antibody is known to be the most prevalent antibody, which interferes with the biological activity of reaginic antibodies, and
helps to confer a certain amount of immunity to the host (Yagi et al., 1963; Perelmutter, 1984).

Pollen of some plants have been considered to be one of the most common causes of seasonal allergic diseases (Bostock, 1819; Schadewaldt, 1967). The clinical manifestation of this allergic response to pollen was called hay fever or pollinosis (Balackley, 1973). Hay fever appears as allergic rhinitis and conjunctivitis. It is characterised by intense sneezing, irritation of the upper respiratory tract, itching of nose and coughing. There is also accompanying conjunctivitis and itchy eyes (Stanley and Linskens, 1974).

While the clinical picture of hay fever is consistent, it was further found that factors on the pollen grains, are the specific allergenic agents. Most pollen grains are fairly large particles, incapable of travelling down the respiratory tract. On fractionation, it has been possible to find the active ingredients from several hundred kinds of allergic plant pollens. Their isolation and purification have been achieved by many research workers (King and Norman, 1962; Underdown and Goodfriend, 1969; Belin, 1972; Viander et al., 1979; Shafiee et al., 1981; Ispén and Lowenstein, 1983; Chakrabarty et al., 1980).
Recently, during the last decade antigenic fragments or haptens have been discovered to trigger immunocytes. Together with these immunocytes, these incomplete antigens are found to induce the antibodies. Their clinical nature, mode of immunological actions and contribution to the overall clinical picture are still to be clarified by further investigation.

In this study the pollen grains of *Prosopis juliflora* have been considered in detail. It is a fast growing shrubby tree, with two very practical advantages:

a) Energy source, by wood waste, and
b) Reclamation of desert lands.

These seem to be justifiable reasons for its large plantation by the help of the Government of India in regions like northern part of India and elsewhere.

The pollen grains of this plant are known to cause allergic diseases (Ordmon, 1950; Bieberdorf and Swing, 1952; Shivpuri and Parkas, 1967).

In the light of the above the extensive plantation of Pj has the following disadvantages:
a) The plant flowers about all year round with peaks in April and September. The allergic nature of its pollen grains and easy dispersal in air, contributes to the overall air pollution load.

b) It poses an insidious threat to the general standards of health and contributes to the overall morbidity.

The primary aim of this study has, therefore been to study the chemical properties of the allergens and the immunological responses, they are capable of eliciting. Therefore, it is proposed to

i) identify and purify the guilty allergic compounds from the pollen grains of Pj;

ii) study the immunochemical responses of its allergens, to grade allergic damage or immune injury;

iii) establish a ratio of allergenicity between the various allergic fractions. Individuals, who are pollen sensitive, have been diagnosed and immunised with specific allergens.

iv) The ratio of allergenicity between fractions will help to put in order a safe and effective dosage regimen, with its concomitant desirable or undesirable side-effects during immunotherapy.
HISTORICAL ACCOUNTS

Our knowledge about hay fever dates back to 1819, when Bostock gave an exact description of the clinical symptoms and later introduced the name "hay fever". Real epidemiological research started with statistical data on prevalence of the illness (Phoebus, 1862; Beard, 1876). Exact scientific method based research started when sources which cause hay fever were established (Stanley and Linsken, 1974; Blackley, 1873). Dunfar (1903) first demonstrated that proteins are the allergenic factors and tried to treat the hay fever patient. Wolffe-Eisner (1907) contributed to the concept that hay fever, in which the hypersensitive reaction to pollen occurs, is an allergic disease. Noon (1911) successfully treated hay fever by desensitizing the patients with pollen injections. Since then many experiments have been carried out to isolate the pollen allergens and to verify the immunochemical nature of pollen allergens (King et al., 1964; Lichtenstein et al., 1973; Underdown and Goodfriend, 1969; Chakrabarty et al., 1982; Yasueda et al., 1983; Ipsen and Lowenstein, 1983; Vela et al., 1982; Creticos et al., 1984).
ALLERGENIC POLLEN SOURCES

About one percent human population of the world suffers from pollinosis caused by pollen of one or several hundred plant species. In India, data for the incidence of pollen allergy is meagre. It is estimated that more than 1 percent of the population of our country suffers from bronchial asthma and 3 to 4 percent from different allergic diseases (Shivpuri, 1967, 1971). Pollen causing allergy are generally grouped as derived from grasses, weeds or trees (Table I). Pollen from genera in the same family or even related species, may differ in capacities for producing allergic reactions (Stanley & Linskens, 1974). Plants which constitute the most important group of pollen causing allergy generally have the following characteristics: (i) wide spread distribution; (ii) easy mode of pollination by wind; (iii) plant must produce large quantities of pollen; (iv) pollen must be buoyant and transportable, and (v) pollen must contain a hay fever allergen.

Respiratory allergy such as hay fever or pollinosis can be caused by pollen contained in the inhaled air in relative abundance which releases a
<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Local name</th>
<th>Season of Pollination and of patient's symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grasses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynodon dactylon</td>
<td>Doob</td>
<td>Major part of the year, particularly autumn and spring.</td>
</tr>
<tr>
<td>Cenchrus ciliaris</td>
<td>Anjan</td>
<td>August-October</td>
</tr>
<tr>
<td>Pennisetum typhoides</td>
<td>Bazra</td>
<td>September-October</td>
</tr>
<tr>
<td>Sorghum vulgare</td>
<td>Jawar</td>
<td>September-October</td>
</tr>
<tr>
<td><strong>Weeds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranthus spinosus</td>
<td>Kantewali</td>
<td>Greater part of the year.</td>
</tr>
<tr>
<td></td>
<td>chanlai</td>
<td>- do -</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>Jangli</td>
<td>- do -</td>
</tr>
<tr>
<td></td>
<td>Bathua</td>
<td>- do -</td>
</tr>
<tr>
<td>Xanthium strumarium</td>
<td>Chhota</td>
<td>September-December</td>
</tr>
<tr>
<td></td>
<td>Gokhru</td>
<td></td>
</tr>
<tr>
<td>Cannabis sativa</td>
<td>Bhang</td>
<td>May-September</td>
</tr>
<tr>
<td>Argemone Mexicana</td>
<td>Satyanasi</td>
<td>January-April</td>
</tr>
<tr>
<td>Brassica compestris</td>
<td>Sarson</td>
<td>December-February</td>
</tr>
<tr>
<td>Cyperus rotundus</td>
<td>Motha</td>
<td>July-October</td>
</tr>
<tr>
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<table>
<thead>
<tr>
<th>Trees</th>
<th>Kabuli</th>
<th>Keekar</th>
<th>Greater part of year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospopsis juliflora</td>
<td></td>
<td></td>
<td>Greater part of year</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td></td>
<td>Arand</td>
<td>October-March</td>
</tr>
<tr>
<td>Salvador persica</td>
<td></td>
<td>Peelu</td>
<td>March-May</td>
</tr>
<tr>
<td>Holoptelia integrifolia</td>
<td></td>
<td>Papri</td>
<td>February-April</td>
</tr>
<tr>
<td>Carica papaya</td>
<td></td>
<td>Papita</td>
<td>October-April</td>
</tr>
<tr>
<td>Putranjiva roxburghii</td>
<td>Putranjiva</td>
<td></td>
<td>April-May</td>
</tr>
<tr>
<td>Morus alba</td>
<td></td>
<td>Shehtul</td>
<td>February-March</td>
</tr>
<tr>
<td>Syzygium cumini</td>
<td>Jamun</td>
<td></td>
<td>April-June</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Neem</td>
<td></td>
<td>April-May</td>
</tr>
<tr>
<td>Terminalia arjuna</td>
<td>Arjun</td>
<td></td>
<td>April-June</td>
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</table>
chemical allergen. According to Witting et al. (1970) these chemical allergens possess following properties: (i) antigen should be foreign to the species; (ii) in general, molecular weight should be over 10,000 daltons; (iii) molecular structure should contain aromatic groups, disulfide linkage or double bonds; (iv) the molecular structure surface configuration should have "polar group" for attaching antibodies and conveying specificity; and (v) should be metabolised by the body in a specific period of time.

Study of *Prosopis juliflora* pollen grains is of significant interest in the present study, because its pollen is one of the major sources of allergic diseases of India (Shivpuri, 1967). *Prosopis juliflora* is a native of arid region of Mexico and Central America. Its pod has high food value as fodder. It can also be eaten by man in time of scarcity. In parts of South West Africa it is so high that authorities have been requested for the periodical removal of these trees (Ordman, 1950). Bieberdorf and Swinny (1952) in Texas carried out routine skin tests in regions where Prosopis trees were abundant and they have reported that 55 percent of patients with respiratory allergy showed positive reaction to Prosopis
Fig. 1: Prosopis juliflora (Kabuli keekar) Plant with Yellow Inflorescence.
pollen extract. Shivpuri (1962) carried out allergy test of Pj pollen in 430 patients suffering from nasobronchial allergy in Delhi and New Delhi areas and reported differences in allergenicity by positive skin reaction test. They also diagnosed allergic patients by giving hyposensitizing dose with antigens that had given significant positive reaction.

Chemical Constituents of Crude Pollen Extract

During the past few years, attempts have been made to characterise the chemical constituents of pollen extract (Gutman, 1972; Ekramoddolullah et al., 1982; Puttonen et al., 1982; Vela et al., 1982; Einarsson and Karlsson, 1982). The most important allergens are considered to be proteins or glycoproteins. Although polysaccharides, lipoproteins, phosphate, sulfhydryl group (-SH) and sialic acid can also be effective allergens. Most of the presently available commercial extracts are characterized by a number of protein-nitrogen units (PNU or Cook units) or the weight to volume (w/v) ratio of the extracted ingredients (Stull and Cook, 1932). A new method and unit of characterisation of allergen extract has been proposed that amount which causes 50 percent
of the extract. Fractionated extracts would permit distinction by dose responsiveness between skin reactive individuals and those with allergic diseases (Turkeltaub et al., 1982).

The most important step in antigen isolation is to establish a reliable quantitative or at least semiquantitative biological assay. The first step in this procedure is to solubilise the antigen, which can then be used to fractionate and analyse antigen. There are many techniques available for fractionation and analysis of antigen based on their physicochemical properties.

i) Gel filtration and ion exchange chromatography are two popularly used methods; the first separation of molecules is based on their shape and size, and the second fractionation of molecules is based on their net electric charge.

ii) Adsorption and partition chromatography are infrequently used methods but can provide elegant separation of antigen. These methods separate molecules based on their affinity or solubility in different substances.
iii) Ultracentrifugation and electrophoresis are frequently used to determine the molecular weight according to charge.

iv) Isoelectric focusing can be used to determine how closely the isoelectric point of the test extract resembles that of the reference standard and thus helps to establish the identity and relative quality of the product.

v) Recently, immunochemical methods have been used. These combine immunologic principles with other methods of separation such as immunoelectrophoresis or immunabsorption.

vi) Quantitative skin testing.

vii) Radioallergosorbent test (RAST) or inhibition test.

viii) Radical immunodiffusion and

ix) Immunoenzymatic test.

These techniques provide high resolution and will be useful to compare one lot of extract with another.

Additional characterization can only be demonstrated after obtaining the maximal activity units per dry weight. It is necessary to demonstrate homogeneity of an antigen. Generally, any of the techniques can be used to characterize homogeneity but it should be
stressed that one must use more than one assay while determining homogeneity of a substance. Also, when using these assays one should demonstrate biological activity contained in a single peak or a single demonstrable band.

Recently, major allergenic molecules of various trees, weeds, and grasses have been isolated and characterized (King et al., 1964; Johanson and March, 1966; Aas and Jøbsen, 1967; Underdown and Goodfriend, 1969; Belin, 1972; Viander et al., 1979; Ceska et al., 1972; Ekramoddoullah et al., 1980; Shafiee et al., 1981; Işıl and Lowenstein, 1983; Yasueda et al., 1983; Baldo et al., 1982). The best known allergenic proteins have been obtained from ragweed, timothy and rye grass, kentucky blue grass, birch, Japanese cedar, Russian thistle, plantain, which is shown in Table II.

Pj pollen has been established as one of the sources of allergy, but impurities and characterisation of active fraction is wanted. Therefore, in this work fractionation, purification and characterisation is of interest.

Very recently haptene like substances were isolated from ragweed and kentucky blue grass pollen
<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Techniques used for characterization</th>
<th>Main Allergens</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birch Pollen</td>
<td>Gel filtration; immunodiffusion; immunoelectrophoresis; Ion-exchange chromatography etc.</td>
<td>Allergens of mol. wt. 20,000</td>
<td>Belin (1972) and Viander et al. (1979)</td>
</tr>
<tr>
<td>Birch Pollen</td>
<td>Crossed radioimmuno-electrophoresis; ionexchange and chelate chromatography; SDS-PAGE; RAST.</td>
<td>Three allergens (Mol. wt. 17,000, 25,000 and 29,000 daltons)</td>
<td>Impén and Lowenstein (1983)</td>
</tr>
<tr>
<td>Japanese cedar</td>
<td>Gel filtration; ionexchange chromatography; immunodiffusion, etc.</td>
<td>SB P-a₁ (mol. wt. 40,000) and SB P-a₂ (mol. wt. 35,000) fractions.</td>
<td>Yasueda et al. (1983)</td>
</tr>
<tr>
<td>Kentucky blue grass</td>
<td>Gel filtration; isoelectric focusing; RAST etc.</td>
<td>Allergen C and C-1-2-6 (mol. wt. 12,000 and 11,000)</td>
<td>Ekramoddoullah et al. (1977)</td>
</tr>
<tr>
<td>Olea europea</td>
<td>Gel filtration; crossed immunoelectrophoresis, RAST etc.</td>
<td>65,000 and 160,000 mol. wt. fractions</td>
<td>Vela et al. (1982)</td>
</tr>
<tr>
<td>Plantain</td>
<td>Crossed immunoelectrophoresis</td>
<td>6 out of 16 allergens</td>
<td>Baldo et al. (1982)</td>
</tr>
</tbody>
</table>

(contd...)
<table>
<thead>
<tr>
<th>Plant</th>
<th>Methods</th>
<th>Antigens/Proteins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragweed</td>
<td>Gel filtration; immunodiffusion</td>
<td>Antigen E, K and Ra&lt;sub&gt;3&lt;/sub&gt;</td>
<td>King et al. (1964)</td>
</tr>
<tr>
<td>Russian thistle</td>
<td>Gel filtration ion exchange</td>
<td>RT&lt;sub&gt;1&lt;/sub&gt; (mol. wt. 39,000)</td>
<td>Shafiee et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>chromatography; SDS-PAGE, etc.</td>
<td>and RT&lt;sub&gt;2&lt;/sub&gt; (mol. wt. 35,000)</td>
<td></td>
</tr>
<tr>
<td>Rye grass</td>
<td>Gel filtration</td>
<td>Group-I, II and III</td>
<td>Marsh et al. (1966)</td>
</tr>
<tr>
<td></td>
<td>Immunoelectrophoresis and crossed</td>
<td>16 out of 32 allergens</td>
<td>Renek and Einarsson (1984)</td>
</tr>
<tr>
<td></td>
<td>radio-immunoelectrophoresis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timothy grass</td>
<td>Gel filtration; immunodiffusion;</td>
<td>Antigen A and B (mol. wt. 13,000 and 10,500)</td>
<td>Malley et al. (1962)</td>
</tr>
<tr>
<td></td>
<td>immunoelectrophoresis etc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gel filtration and RAST</td>
<td>Glycoprotein (mol. wt. 25,000)</td>
<td>Puttonen et al. (1982)</td>
</tr>
</tbody>
</table>

Molecular weight is expressed in daltons.

SBP-<sub>a</sub><sub>1</sub>, SBP<sub>a</sub><sub>2</sub>, RT<sub>1</sub>, RT<sub>2</sub> and C-1-2-6 are types of allergens.
by using gel filtration and preparative isoelectro-focussing which could provide protection from allergic reactions (Attallah and Sehon, 1969; Chakrabarty et al., 1980). Therefore in the present study passive Cutaneous Anaphylaxis test and Radioallergosorbent test of fractionated pollen of Pj were carried out to know whether haptene like substances exists in Pj pollen grains. The significance of allergens have been proved and awaits further chemical characterization. Further recognition and comparisons of nature of human responses in pollinosis and its desensitization therapy can be improved.

PATHOGENESIS OF ALLERGENS OF POLLEN GRAINS

In 1913, Dale postulated that the allergic responses involve an immunological complexing reaction between an allergen and an antibody. The complex induces the detrimental allergenic reaction recognized as pollinosis. Allergic reactions occur in different organs or tissues. If the lower respiratory tract, lungs, or bronchi are affected, the clinical reaction will be that of asthma. Other reacting organs may be the skin (Urticaria), the gastro-intestinal tract, the central and peripheral nervous
system or the cardiovascular system. The organ involved in the allergic reaction is called "shock" organ (Stanley and Linskens, 1974).

I. Genesis of Allergenic Reaction of Pollen Grains:

Pollinosis may be caused by following methods (Stanley and Linskens, 1974).

1. Pollen enters the nose or eyes and lands on mucous membrane tissues of the upper or lower respiratory tract;

2. Mucous liquid solubilizes pollen allergen which penetrate the mucous tissues;

3. Mucous tissues of allergic persons contain mast cells which have a high concentration of antibodies;

4. The antigen, e.g., pollen allergens quickly complex with IgE or IgG type of immunoglobulins;

5. The complex activates enzymes which cause the release of mediators from special organelles in these cells;

6. The chemical mediators, e.g., histamine induce the allergic symptoms, i.e., dilation of blood capillaries, contraction of muscles, oedema of mucous
membrane, hyper reaction of watery nasal fluid and constriction of nasal or bronchial passages— the hay fever reaction.

II. Immunological Mechanism of Allergic Reaction:

Pirquet (1906) defined allergy very precisely as the acquired specific altered immunological capacity to react. Immunity is acquired when a previous exposure to the allergen stimulates the immune system and develops hypersensitivity. "Specific" refers to the very precise molecular relationship existing between allergen and the corresponding antibodies produced in the response.

The allergic response may be increased as in hypersensitivity or it may be decreased as a result of increased immunity. The specific immune response consists of two effector mechanisms (a) the production of antibodies by bone marrow dependent B-lymphocytes and (b) lymphocytes mediated elimination, manifested by the thymus dependent T-lymphocytes. It also continues to persist in tertiary responses, which are not beneficial to the host. It causes different allergic diseases, but whether it is due to antigen persistence and the nature of antigen itself or
of genetic or maturational defect of host is not clear (Fig. 2).

There is well established evidence for the role of type-I reaction in allergic diseases. More recently, evidences of type-III reaction in allergic diseases have been reported (Pepys 1973).

1. **Immediate Hypersensitivity (Type-I) Reaction**

Type-I reactions are those in which antigen reacts with tissues or cells passively. They are sensitized by antibodies and induce the liberation of pharmacologically active mediators. All mammalian species are capable of producing systemic, local and **in vitro** anaphylactic reactions. These immunoglobulins which cause these reaction have been referred as "anaphylactic antibodies", "skin sensitizing antibody" and "reaginic antibody". Generally, these antibodies are "homocytotropic", a term introduced by Becker and Austen (1966). Two types of homocytotropic antibodies have been identified. These are IgG\textsubscript{1} and IgE detected in most of the mammalian species. There is general agreement that both occur in guinea pigs, rats, mice, dogs and monkeys (Bloch 1967).
Fig. 2: Schematic summary of the cellular and immunological interactions involved in allergic responses.
2. **Type-III Reaction**

Complex mediated hypersensitivity reactions result from the effects of antigen-antibody complexes i.e. (a) activation of complement and attraction of polymorphonuclear leucocytes which release tissue-damaging enzymes on contact with the complex, and (b) aggregation of platelets to cause microthrombi and vasoactive amines release (Coombs and Gell, 1968). Experimentally, local inflammatory (Arthus) reactions are observed when an antigen is introduced into the dermis of an immunized animal (Humphrey and White, 1970). The necessity for activation of the complement sequence is shown by the fact that in guinea pig IgG\(_2\) antibody activates the complement and initiates Arthus reaction. Activation of complement gives rise to granules in mast cells and enzyme released can produce inflammatory changes and degrade connective tissues (Henson, 1970; Henson and Cochran, 1974). IgG\(_d\) antibody, however, fails to activate the complement.

3. **Interaction of IgE and IgG Antibodies**

Immunoglobulins and sensitized lymphocytes play a vital role in immunopathological responses of body systems. These responses are seldom limited to a
single class or type of antibody. Indeed, the final expression of immunity or hypersensitivity is usually determined by the interaction of the participating factors. The homocytotropic antibody seems to exaggerate the allergic reaction; perhaps by releasing histamine and other mediators and causing increased inflammation (Askenase, 1973; Henson and Cochrane, 1971).

Antibodies both IgG and IgE bound to specific receptors on basophils interact with antigen and induce the release of granule associated as well as newly synthesized mediators (Ovary et al., 1963; Lichtenstein et al., 1978). It is also suggested that basophils also participate in certain delayed hypersensitivity reactions (Askenase, 1977; Dvorak, 1976), and the term cutaneous basophilic hypersensitivity (CBH) has been used to describe these responses (Richerson et al., 1970). Interaction between IgG and IgE antibodies have been proposed in human serum sickness (Zvaifler, 1975).

IgG antibody may alternatively interfere with the biological activity of homocytotropic (IgE) antibody. This is presumably the basis of the hyposensitization therapy used for extrinsic asthma and hay fever.
Repeated small injections of immunogens lead to the production of blocking the antibody of the IgG class (Yagi et al., 1963). Passively, blocking antibody suppress the synthesis of IgE antibody (Stanworth and Smith, 1973; Shalkif and Stanworth, 1980; Perelmutter, 1984). There is evidence for a feed back regulation of homocytotropic antibody formation by passively administered IgG antibody (Lichtenstein et al., 1978; Johansson, 1980). Therefore, it is proposed to study type I and type III reactions in guinea pigs by skin reaction tests.

**Guinea Pig: As Experimental Animal’s Model**

Anaphylactic type of immediate hypersensitivity has been studied in several animal species (Ishizaka and Ishizaka, 1973; Stechschulte, 1978; Hong, 1978; Hook, 1984). However, guinea pig as animal’s model for the study of allergic reaction has certain relative advantage over other animals.

(i) Sensitization is easiest to induce in guinea pig in comparison to rat, chicken etc. (Storck, 1962).

(ii) Guinea pig requires less than 1 μg of protein for generation anaphylactic reactions, which is independent of an intact complement system whereas
other animals, viz. rat, rabbit, require more μg quantities for anaphylaxis.

(iii) It is characteristic of the guinea pig that it produces a very small amount of circulating antibodies against a foreign protein, unlike the chicken and rabbit.

(iv) Certain hereditary characteristics can play a part in sensitization in guinea pigs and that are related to man (Chase, 1941).

(v) There is resemblances in allergic reactions of the guinea pig and man in the histological examination (Bloch et al., 1931).

(vi) Recent evidences suggest basophils participation in cutaneous basophilic hypersensitivity (CBH) which is elicited by T-cells or antibodies in guinea pigs. It is suggested that this cellular hypersensitivity plays an important role in immunologic pathogenesis and desensitization (Richerson et al., 1970; Moore, 1982).

In view of the above fact, it seems that guinea pig is suitable for study in allergic diseases. Male albino guinea pigs have been used in the present work because the macroscopic and microscopic reactions are easier to observe than in pigmented skin; male in order not to be bothered by the oestrogenic cycle or by gestation.
Testing Reactions to Pollen Allergens

In the present study, testing methods were chosen according to specificity, sensitivity, reproductivity, accuracy, rapidity of response, technical requirements, qualitative value, quantitative value, price and limitation of laboratory conditions.

The most common methods for testing pollen allergens are by dermal tests, i.e., prick and intracutaneous skin tests (Blackley, 1880; Rappaport and Becker, 1949; Voorhorst et al., 1973; Solley et al., 1976), and histamine release by leucocytes (Lichtenstein and Osler, 1964; May et al., 1970; Lett-Brown et al., 1981; Tanizaki et al., 1984; Hook et al., 1984). Allergic skin testing is indicated in those individuals in whom homocytotropic antibodies mediate immune response. Cutaneous tests have the advantage because of their simplicity, painlessness, and can be well correlated with other tests, i.e., histamine release test and RAST inhibition test (Indrajana, 1971; Ellis, 1978; Turkeltaub et al., 1984).

The appearance of immediate and late effects are the typical dermal manifestation of the interaction of allergen with mast cells (Ellis, 1978) (Fig. 3), cause the release of vasoactive amines. Important amines are histamine and serotonin, a factor which is
BIRTH OF AN ALLERGIC REACTION

White blood cell mass-produces IgE antibodies after exposure to allergens.

IgE antibodies

Allergens bridge up to 500,000 IgE molecules on individual mast cells, pulling them together.

Histamine

The bridged IgE molecules stimulate the mast cell, causing the release of histamine and other chemicals.

Capillary

Increased capillary permeability and dilatation

Wheal and Flare

Histamine, acting on small vessels in the skin, causes increased permeability and dilation resulting in wheal formation.

Fig. 3: Allergy skin test. Antigen (Allergen) by interacting with cell-bound specific reaginic antibody, activates a biochemical reaction resulting in histamine secretion from mast cell granules. Histamine, acting on small vessels in the skin, causes increased permeability and dilation resulting in wheal formation.
chemotactic for eosinophils (ECF-A), a potent platelet activating factor (PAF), leucocyte kallikrein and the slow reacting substances of anaphylaxis (SRS-A) (Henson, 1978). Histamine is most active among all these vasoactive amines. It is formed by the decarboxylation of L-histidine by a specific L-histidine decarboxylase enzyme. It acts on small blood vessels at the site of the reaction and causes dilation and increased permeability, resulting in localized edema recognized as wheal reaction (Solley et al., 1976; Golub and Golub, 1978; Hook et al., 1984). Histamine release test has certain advantage with precise, reproducible laboratory tests that correlate well with the patients' clinical allergic tests. Leucocytes histamine release test was considered to be largely a research tool because it was both laborious and time consuming.

Further exploration of mechanisms of histamine release suggests that cyclic AMP modulates the anaphylactic release of mediators from mast cells (Bourne, et al., 1974; Orange et al., 1971). Goldberg et al. (1975) have suggested that cyclic AMP and cyclic GMP have opposing effects on cell function and acts jointly as messengers in cells.
With acute allergic reactions, the *in vivo*

situation is complicated because:

i) multiple hormone receptor interaction require
    cellular integration of many signals;

ii) humoral and cell-associated enzymes destroy
    hormones;

iii) after a definite time period cell may become
desensitized, e.g., by inactivation of their hormone
    receptors (Perper *et al*., 1972; Kram *et al*., 1975).
This is the principal reason that amine-inhibitors are
useful in the therapy of pollen allergy.

**Immunochemoical Responses of Allergens of Pollen Grains**

Immunological *in vitro* quantitation of allergen
is highly desirable (Belin, 1972) for their specificity
and ease of study.

*In vitro* immunological techniques are widely
used for characterization and quantitation of antigen
and antibodies. They are easy to perform, give extrem-
ely valuable information and do not require elaborate
equipment or reagents. These techniques are based on
the ability of antibodies to perform precipitin lines
specifically with the antigen (Williams and Wilson,
1981). Both qualitative and quantitative analysis.
i.e., gel-diffusion test; immunoelectrophoresis; crossed radioimmunoelectrophoresis; radioalloserorbent test (RAST), immunoenzymatic assay, have been performed in recent years (Weir, 1973).

For the assessment of in vivo pollen allergen activity, skin test is time consuming, relatively inaccurate and ultimately depends on sufficient sensitized subjects.

In vivo passive cutaneous Anaphylaxis tests have been performed in recent years (Attallah and Sehon, 1969; Chakrabarty et al., 1980). These tests measure the reactivity of a patient's serum to the total antigen components of an extract without knowing what those components are. The separated antigen components and reactivity with antiserum will clearly indicate the allergenicity of particular allergen (antigen). The use of allergen will improve the accuracy and reliability in diagnosis and treatment. Such extracts would permit distinction by dose responsiveness, between skin reactive individual and those with allergic diseases (Ekramoddoullah et al., 1981; Puttonen et al., 1982).
Recently, immunoreponses of major allergenic molecules have been tested (King et al., 1964; Aas & Jeb- sen, 1967; Belin, 1972; Viander et al., 1979; Chakrabarty et al., 1980; Shafiee et al., 1981; Yasueda et al., 1983; Renek and Einarsson, 1984), to standardize crude allergenic extracts for quantitation in mass units of each purified allergen.

In the present study, attempt has been made to isolate the allergen extracts from the Prosopis juliflora pollen grains. Further, its allergens or antigens were purified and standardized by various physiological and immunological techniques. This study might provide clue to understand the mechanism of pollen allergy and basis for the use of allergens in immunotherapy.