A CLINICAL EVALUATION OF THE RAST PROCEDURE FOR IgE ANTIBODIES

by

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ABSTRACT

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A large number of people in this country are afflicted with allergic disorders of varying degrees of severity. These disorders can range from mild hayfever to severe asthmatic conditions. Two chemical entities involved in the manifestation of these disorders are the antigens and the antibodies.

The antigens, like pollen, stimulate the production of antibodies to form antigen-antibody complexes. The antibody primarily responsible for the allergic response is called Immunoglobulin E, IgE. The exact mechanism of action of the IgE is unknown at this time, but it is thought that it attaches itself to the cells in the body's connective tissue and stimulates the production of various chemical mediators. One of the prime chemical mediators is histamine which is the agent primarily responsible for the stuffy nose and congestion associated with allergies.

The diagnosis of allergies is accomplished through the use of a clinical history and various diagnostic methods. Two diagnostic methods commonly used are skin and provocation testing. Both of these

test types are time consuming, inconvenient, uncomfortable and in the case of provocation testing, potentially harmful. The Radioallergosorbent Test (RAST) and the Radioimmunosorbent Test (RIST) are two new analytical tools that show great promise in allergy diagnosis. Both techniques involve the measurement of allergen specific or total IgE by the use of ^{125}I coupled to either an anti-IgE or IgE-^{125}I respectively and the measurement of the resulting radioactivity.

The evaluations conducted at YSU were done on twenty-four patients of a local allergist and seven volunteers from YSU and the Youngstown Hospital Association. The RAST was performed on all patients utilizing a combination of eight possible allergens and the RIST was performed on seven patients.

The results of the RAST were compared with information received from the allergist which included clinical comments and skin test evaluations. Four of the eight allergens were used and two of the four had a regression analysis done on them. The YSU correlation results were compared with literature values. It was found that the RAST results compared very favorably.

The RIST results were also compared with literature values. While the values arrived at by YSU varied widely, they were within normal ranges.

A brief cost analysis showed that the RAST and RIST compared favorably with the costs of other laboratory tests and might be implemented in the series of tests possible in a clinical laboratory.

As a result of this evaluation, the RAST and RIST can be seen to be a valuable diagnostic tool in allergy evaluations. The technique is not difficult or overly expensive and can serve as a valuable addition to the techniques available for the diagnosis of allergies.

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LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS
IgE	Immunoglobulin E	-
^{125}I	Radioactively labeled iodine	-
IBM	International Business Machines	-
l	Liter	-
ml	Milliliter	10^{-3} l
MCR	Mean count rate	-
MCR_o	Mean count rate of zero tubes	-
μ l	Microliter	10^{-6} l
N	Normal solution	equivalent /l
ng	Nanograms	10^{-9} grams
%	Percentage	parts in 100
Phadebas	Registered Trademark of Pharmacia Laboratories Pharmacia determination of biologically active substances	-
RAST	Radioallergosorbent test	-
RIST	Radioimmunosorbent test	-
rpm	Revolutions per minute	-
SD	Standard deviation	-
STAT/BASIC	A set of statistical programs in BASIC language used in conjunction with the IBM Computer	-
U	Unit of IgE	
w/v	Weight per volume	g/100 ml
WHO	World Health Organization of the United Nations	-

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CHAPTER I

BACKGROUND AND INTRODUCTION

Allergies

Allergies are not new. The first description of hayfever and asthma occurred in about 1565. The word allergy is a combination of two Greek words; allo meaning different and ergon meaning action. The earliest description of food allergies is thought to have occurred as early as 65 BC.

Millions of people suffer from allergies. It has been estimated that about half of the population of the world suffers from some sort of allergy, either mild or serious. In the United States, this figure would be in excess of 100 million people. The number three chronic diseases in the world are hayfever and asthma. One third of all chronic childhood diseases are caused by asthma, hayfever or other allergic disorders. Allergies account for the loss of more than 184 million man-days a year. The dollar cost would be in excess of 235 million dollars a year.[1]

An allergy is an altered reaction of tissues in certain individuals upon exposure to agents which in similar amounts do not affect other persons. Allergies occur in two forms; the delayed and immediate type. In the delayed type, antibodies do not seem to be required. It is caused by antigens but a very minimal

amount of information is available about their mechanism of action. The immediate type is caused by antigens(i.e. allergens) which stimulate the production of antibodies within a given individual. Several examples of the immediate type are hay fever,asthma and hives.

Allergies can be classified into six groups:[2]

1. Inhalants- A most important allergen causing symptoms in the respiratory tract. These allergens include pollens,dusts, vapors,animal dander,(i.e. perfume and various other odors).

2. Food-Food is a very important allergen. Any food may be a possible offender but the most common ones are wheat,eggs,milk, fish,chocolate and strawberries.

3. Drugs-This is a third category and deals with medications like penicillin and sulfa drugs.

4. Infectious agents-Bacteria,fungi,parasites and several viruses are very capable of producing tissue sensitivity.

5. Contactants-These act on the skin and mucous membranes causing an irritation of these membranes. Common examples are poison ivy,poison oak,and poison sumac which cause very fine rashes.

6. Physical agents-The chief physical agents are heat,cold, light,and pressure. The result can be seen in the respiratory tract or the skin.

While the number of categories of allergies is confusing, there are several different types of allergies. The most common type of allergy is probably allergic rhinitis which is characterized by sneezing, runny nose, and itchy eyes. Allergic rhinitis occurs in two forms. The seasonal form, hay fever, is generally induced by wind-borne pollens or fungi. The other form is perennial allergic rhinitis in which the individual has symptoms year round but in varying degrees of severity.

Bronchial asthma characterized by wheezing due to the narrowing of bronchial passages is usually found in individuals with an inherited allergic constitution. Extrinsic asthma is due to various external factors like pollen or molds while an internal or intrinsic asthma is due to an infection in the upper or lower respiratory tract.

A third type, gastrointestinal allergy, is ordinarily due to the ingestion of food allergens. The chief effects are nausea, vomiting, abdominal cramps, and diarrhea.

In hives, called urticaria, the result is a wheal rising on the skin. The single most important cause of this allergy type would seem to be food allergies, but physical allergies have been known to cause hives.

There are three other allergy types and while they cannot be considered major types will be included for completeness. One of these is called serum sickness and is an allergic reaction appearing eight to twelve days after the administration of a foreign serum.

It is characterized by fever,skin eruptions,and even an arthritic condition. Similar to serum sickness are the drug reactions caused by the administration of a drug. The symptoms of the drug reaction can range from headache to nausea and diarrhea. The last category is called anaphylactic shock. This is a severe, often fatal reaction. In severe anaphylactic shock,changes are seen in the lungs and symptoms occur almost immediately. The symptoms in the milder form are sneezing and coughing while in the more severe cases,convulsions and eventually death are possible. One of the most common causes of this type of reaction are stings of wasps and bees.

Allergy Diagnosis [3]

When allergies are suspected in an individual,a series of procedures are utilized to see if an individual is allergic and to what specific allergens.

The first step is a detailed history. The time or season of attacks(i.e. fall,summer), character of the attacks,frequency and possible precipitating factors must all be determined. Also, how the individual obtains relief may give an idea as to the cause. Data about occupation and location of employment must be noted. In children,any food that is intensely disliked should be taken as indicative. The dislikes of children tend to be protective rather than just whims. Various other environmental factors such as the type of pillow the person uses,exposure to animals and the effect of outdoor work should also be determined.

The next step and the one with which most allergic people are familiar are the skin tests. These are very useful in allergies caused by contactants and inhalants. The wheal and flare,similar to a large mosquito bite,is a positive response. This response occurs within about 5-15 minutes. Skin tests are of three general types; (1) scratch tests which are performed in rows on the patients back or forearm. The scratches are about one cm. long and about 2.5 cm. apart. The skin is torn rather than cut with no blood drawn. An allergen of interest is then applied to each scratch. (2) In intradermal testing,a tuberculin syringe fitted with a short needle is used. About 0.02 ml. of the allergen of interest is injected. A separate syringe and needle is used for each allergen. (3) Patch tests entail the application of the allergen to a patch of linen or gauze. The patch is then applied to the skin by adhesive or cellophane tape. The patch should remain for 24-48 hours unless irritation occurs.

Mucosal provocation tests are utilized when skin tests yield negative results and there is clinical evidence to the contrary. The procedure entails the utilization of 1.0 ml. of an allergen extract inhaled through a nebulizer. In absence of a bronchial response,the procedure is repeated at 10 minute intervals increasing the concentration of the allergen tenfold until a positive reaction is obtained or the most concentrated allergen extract is reached. In nasal provocation testing, the allergen extracts are used in 0.05 ml. quantities and administered with a 1 ml. disposable syringe. In the absence of a reaction, the procedure is repeated at 10 minute intervals increasing the

allergen concentration tenfold until either a positive reaction occurs(i.e. sneezing,itching or blocking of the nose) or the highest concentration is reached. The reactions are evaluated 10 minutes after allergen administration.[4]

In the realm of food allergies,food diaries and elimination diets are used. In the food diary,the foods eaten are listed and the date symptoms appear also is listed. This allows the determination of the possible offending agent. The elimination diet consists of a greatly restricted intake of foods. Only certain foods are eaten,at first and if no reaction occurs then another food is added to the diet. When symptoms occur, the particular food that might be the possible cause can be tracked down and eliminated.

The blood eosinophil count is elevated in many individuals during allergic attacks and can be used as clinical evidence for possible allergies if correlated to a positive response from other tests or clinical symptoms. The eosinophil is a granular leukocyte believed to be produced in the bone marrow, released into circulation,and attracted to tissues. The ratio of eosinophils in blood,bone marrow and tissue is about 1:200:500.[5]

Antigen

The definition of an antigen is largely functional. It is considered to be a substance that when introduced into the system stimulates antibody formation by attachment to a carrier macromolecule. This definition is also relative since the response is very often a property of the route of injection, method of

preparation of the antigen and the antigen species used. The term hapten is taken from the Greek verb meaning to touch, to grasp or to fasten. This is a most descriptive definition of the process of the binding of an antigenic determininant to an antibody binding site. To emballish on this simple definition, a hapten is that specific chemical grouping to which a single antibody site conforms and reacts.[6]

There are some general characteristics that can be applied to determine the antigenicity of a molecule.[7]

1. For a molecule to be antigenic it must be foreign and the more foreign the more antigenic.

2. A certain molecular weight is necessary. The smallest antigen is glucagon with a molecular weight of about 4000. Insulin with a molecular weight of about 6000 is also antigenic, but certain molecules with molecular weights of about 10,000 are non-antigenic. Several examples of this type molecule are lysozyme and certain protamines.[8]

3. If a molecule is large it cannot be said that it is necessarily antigenic. A rigid structure would seem to be a prerequisite for antigenicity.The type and arrangement of the polar groups on the molecule are very important in determining the specificity of an antigen. Gelatin resulting from the partial hyrolysis of collagen is non-antigenic but it has been shown that with the attachment of L-tyrosine or L-phenylalanine to gelatin the resulting molecule becomes very antigenic. It is thought that the attachment of these groups tends to increase the rigidity of the molecule.[9]

4. An antigen must be easily digestible, but at the same time must not be broken down too readily. The antigenicity of a molecule depends on it being in the animal in the unaltered state for a period of time.

Antibody

The other of the entities in allergies are the antibodies. The antibodies in man and animals belong to a category of proteins called immunoglobulins. These immunoglobulins sometimes called gamma globulins are a component of plasma protein. They are defined as that component of plasma protein that migrates the most slowly during electrophoresis at alkaline pH. Two symbols, Ig and γ, are used for the immunoglobulins and are followed by a capital letter to designate the particular class. The immunoglobulins are divided into three major and two minor classes. The three major classes are IgG, IgA and IgM with the two minor classes being IgD and IgE.

IgG is the major fraction of the immunoglobulins and accounts for about 85% of the total. About 5% of the total gamma globulin is the IgM fraction. The protein which composes about 10% of the immunoglobulins in human serum is IgA. About 1% of the total is IgD while about .01% of the fraction is the IgE portion.[10]

The human immunoglobulins can be split into three fragments with the monomeric enzymes papain and trypsin. Figure 1 shows the cleavage of IgG by papain. Two of the fragments are identical and designated F_{ab} (fragment, antigen binding). The third fragment can be separated from the other two by crystallization, column chromatography, or electrophoresis and is designated

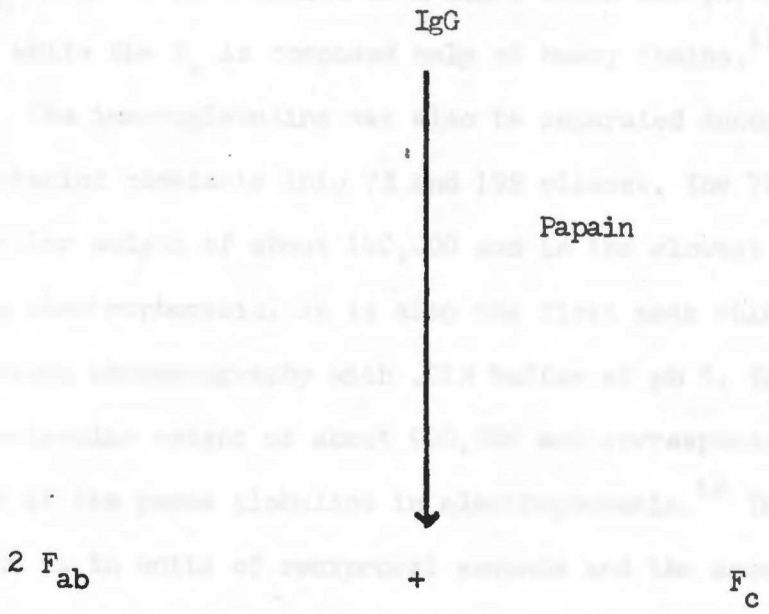


Fig. 1.-- Proteolytic Cleavage of Immunoglobulin G (IgG)

F_c (fragment, crystallizable). Each of the immunoglobulins has two identical F_{ab} and one F_c fragment. Upon exposure to cysteine they yield two types of polypeptides: the heavy chain (MW 50,000) and the light chain (MW 25,000). Experimental evidence has shown that the F_{ab} fragment is composed of a light chain and portions of heavy chains while the F_c is composed only of heavy chains.[11]

The immunoglobulins can also be separated according to their sedimentation constants into 7S and 19S classes. The 7S fraction has a molecular weight of about 140,000 and is the slowest migrating fragment in electrophoresis. It is also the first peak which elutes from DEAE column chromatography with .01M buffer at pH 8. The 19S fraction has a molecular weight of about 900,000 and corresponds to the faster portion of the gamma globulins in electrophoresis.[12] The sedimentation constant is in units of reciprocal seconds and the amount 10^{-13} seconds is taken as one Svedberg (S). This constant is used in the calculation of molecular weight using a form of the Svedberg equation.[13]

$$M = \frac{RTS}{D\,(1 - \bar{v}\rho)}$$

M = molecular weight
T = absolute temperature
D = diffusion constant
D = RT/f
R = gas constant
f = frictional coefficient
S = sedimentation constant
$\bar{v}$ = partial specific volume
ρ = density

Each immunoglobulin has a unique heavy chain. These are designated $\alpha, \gamma, \mu, \delta$ and ϵ for IgA, IgG, IgM, IgD and IgE respectively.

There are two different light chains possible designated Type K or Type Kappa, κ and Type L or Type Lambda, λ. For all immunoglobulins, the amount of Type K is about 60% and Type L about 30% the remainder being nonspecific. The properties of the immunoglobulin classes are

summarized in Table 1.

The role of IgE in the allergic response is somewhat cloudy, but Ishizaka showed that in the allergic patient, the presence of allergen specific IgE attached to the surface of the mast cell is a prerequisite for the development of the immediate hypersensitivity reaction.[15] The mast cells are large round or ovoid cells found in loose connective tissue. The IgE provides a mechanism for concentrating the allergen on the surface of these cells. Amines like histamine are released from the sensitized cells and produce a localized anaphylaxis in the skin or mucosal linings of various parts of the body. This anaphylaxis can be in the form of swollen nasal passages, itchy eyes and swollen bronchial passages. The mechanism by which this release occurs is obscure but is thought to deal with some cellular factors, possibly enzymes or hormones.

The formation of IgE seems to occur only in deposits of IgE producers in the respiratory and gastrointestinal mucosa, lymphatic nodes, tonsils, adenoids and bronchial passages.[16]

The physiochemical properties of IgE show that it has the general features in common with other immunoglobulins. It consists of two kinds of polypeptides, the light and heavy chains. It additionally consists of four single chains linked by disulfide bonds and noncovalent forces. This protein migrates in the γ region in electrophoresis and is eluted from Sephadex G-200 with IgA. The γ region is that region which is closest to the origin in electrophoresis.[17] Even though IgE and IgA are eluted close together, IgE appears earlier and forms a narrower peak. It has a carbohydrate content of 10.7% which is much higher than that of IgA. The isolation of the polypeptide

TABLE 1

PROPERTIES OF IMMUNOGLOBULIN CLASSES [14]

WHO Nomenclature	Serum Concentration(mg/ml)	Molecular Weight	Sedimentation Coefficient
IgG	12	150,000	7
IgA	3	180,000-500,000	7,10,13
IgM	1	950,000	18-20
IgD	0.1	175,000	7
IgE	0.001	200,000	8

chains requires that the interchain bonds are cleaved prior to separation of the chains in dissociating media.[18] If mild conditions are used the chains will retain their antigenic character.[19] Since the light chains of all immunoglobulins are alike, the heavy chain is of considerable importance. This chain includes a covalently linked carbohydrate group and accounts for the high molecular weight of IgE. This chain is considerably larger than the heavy chains of the other immunoglubulins the difference being about 100 amino acid residues. The other immunoglobulin heavy chains are about 400 amino acid residues in length. The IgE antibody activity is inactivated completely in one hour at 56°C. Trace amounts of IgE are present in the serum. Elevated levels of IgE can be seen in patients with certain allergies.

Antigen-Antibody Reaction

There are two visible reactions resulting from the interaction of antigen with antibody. These two reactions are the precipitin reaction, if the antigen is in soluble form and agglutination, if the antigen is particulate. The visible clumping occurs with most antigens because the antigen is multivalent and the antibodies are thought to be at least bivalent, therefore large antigen-antibody aggregates are formed. The two reactions are reversible and can be carried out under essentially equilibrium conditions. It is thought possible to make three generalizations about antigen-antibody reactions:[21]

1. Under normal conditions, antibodies are produced only in response to antigen stimulation. This generalization serves to separate antibodies from other binding proteins in the serum such as transferrin, a

TABLE 2

AMINO ACID COMPOSITION OF IMMUNOGLOBULIN E [20]

Substance	Residues per 190,000 grams	Percentage of moles
Tryptophan	37.5	2.5
Lysine	62.3	4.1
Histidine	29.9	2.0
Arginine	68.6	4.5
Aspartic Acid	111.2	7.3
Threonine	169.5	11.2
Serine	186.1	12.3
Glutamic Acid	131.5	8.7
Proline	102.6	6.8
Glycine	104.3	6.9
Alanine	104.9	7.0
Half-cystine	40.0	2.6
Valine	107.2	7.1
Methionine	16.9	1.1
Isoleucine	38.6	2.5
Leucine	102.2	6.7
Tyrosine	54.0	3.6
Phenylalanine	50.6	3.3
N-acetylglucosamine	36	

beta globulin of molecular weight about 85,000 which is capable of combining with iron, zinc, and copper. It's primary function is to transport iron.

2. Antibodies are heterogeneous not only in regards to structure but also with respect to the bond they form with the corresponding antigen.

3. All antibodies have the capacity to bind with their respective antigens. This binding is to the F_{ab} fragment discussed earlier.

To understand the reaction between antigen and antibody one must understand affinity and to understand affinity one must understand the "Law of Mass Action". This is the intrinsic reversible reaction that leads to complex formation between antigen and antibody. Affinity is the "pivotal element in the biological activity of a molecule".[22]

Consider the interaction between an individual antibody site, F and an individual antigen site, H to form a joined unit, FH. With forward and reverse reaction constants of k_1 and k_2, respectively, the reaction can be written in a simple bimolecular form.

$$F + H \underset{k_2}{\overset{k_1}{\rightleftharpoons}} FH \qquad (2)$$

Chemical affinity is influenced by two factors, a concentration effect and a specific affinity which depends on the chemical nature of the reacting substances, their temperature and pressure.[23]

The antibody, like many enzymes depends upon its primary structure to specify the binding sites. Although the active site properties of antigen and enzyme seem closely related there are

two general characteristics that distinguish them. A major distinction is that antibodies don't ordinarily participate in synthetic and degradation processes. The other distinction deals with the fact that enzymes are usually regarded as univalent while antibodies possess more than two binding sites per molecule.[24]

If an unsensitized individual is injected with an antigen, he responds by producing a low level of detectable antibody in five to seven days. The level or titer of this antibody rises slowly and reaches a peak in from three to six weeks. After this time the level drops off and reaches a low level again. This is termed the primary response. If at this time more antigen of the same kind is reinjected there will be an immediate although short-lived drop in the titer. This drop will be essentially a neutralization reaction. This will now be followed by a very rapid rise in the titer. The slope of the resulting curve will be much steeper and the level of the antibody is at a higher peak and this high level is maintained for a period of time. These responses are summarized in Figure 2.[25]

The antigen-antibody reactions bring about the release of certain chemical mediators which in turn bring about tissue changes. Each of these mediators will be discussed briefly.

The best known of these is histamine (β-imidazolylethylamine). It seems to be most responsible for many allergic manifestations. The histamine is bound to heparin in the mast cells. Heparin with a molecular weight of about 17,000 prevents the coagulation of plasma. Upon disruption of the mast cells, histamine is liberated. The histamine then causes the disruption of the integrity of the capillary

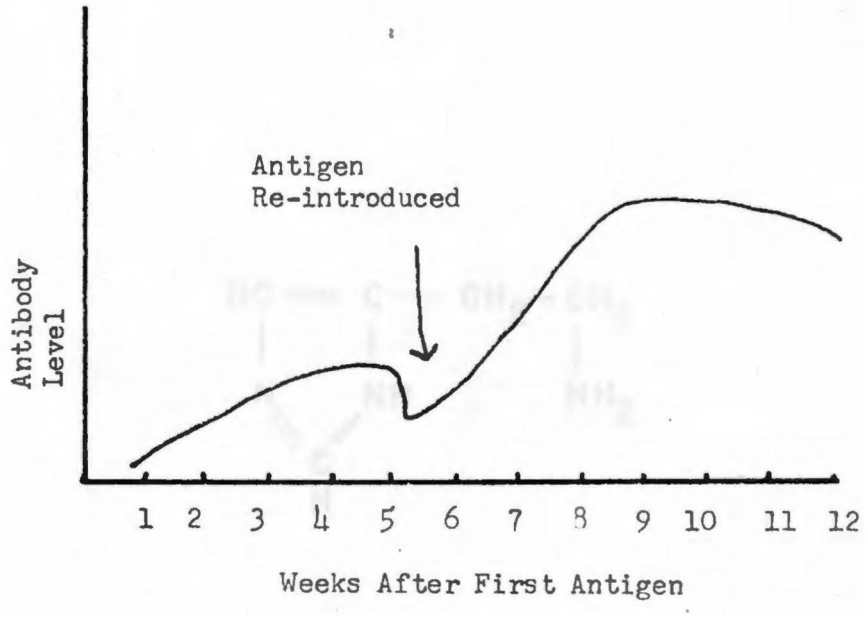


Fig. 2 Summary of the Allergic Response

walls which results in the swelling of the mucous membranes. This then causes the contraction of the smooth muscles which includes the bronchial passages. The chemical structure for histamine is seen in Figure 3, while the postulated repeat unit for heparin is in Figure 4 .

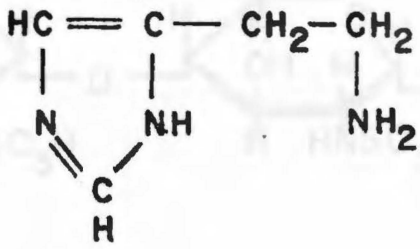


Fig. 3-- Histamine

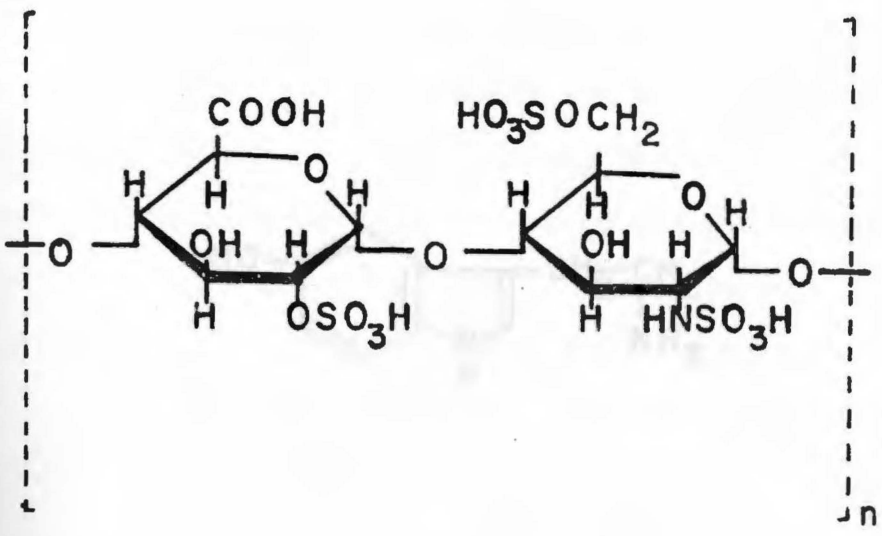


Fig.4-- Postulated Repeat Unit
for Heparin

Another of the mediators is serotonin (5-hydroxytryptamine). It appears to be liberated with histamine and mimics some of the tissue responses which result from histamine. Serotonin is a constituent of several wasp venoms and N-methylated derivatives can cause central nervous system damage. Figure 5 shows the chemical structure for serotonin.

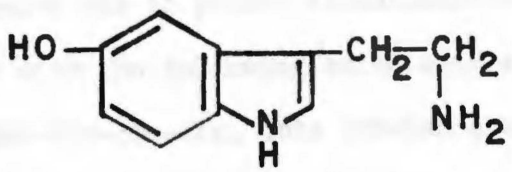


Fig.5-- Serotonin

SRS-A or slow reacting substance of anaphylaxis is a chemical substance whose structure has not yet been elucidated. It is gradually released from lung tissue and produces slow and protracted contractions of bronchioles during asthma attacks. It appears to demonstrate smooth muscle contracting activity and increase capillary permeability. Studies have shown SRS-A to be an acidic,low molecular weight substance active at the nanogram level or less. It additionally,is very resistant to a variety of proteolytic enzymes,phospholipases and neuraminidase.

Another mediator is bradykinin. Bradykinin is one of the plasma kinins which increase capillary permeability as well as being hypotensive due to potent vasodilator action. Bradykinin is a nonapeptide with the following amino acid sequence: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. This substance and another called kallidin arise from the proteolysis of the globulin fraction of plasma known as kinogen.

The mediator ECF-A,eosinophil chemotactic factor for anaphylaxis is released from sensitized human lung fragments when they are challenged with antigen. It appears to be a peptide of molecular weight 1000,which attracts eosinophils.

Initial studies have shown that prostaglandins were released from sensitized guinea pig lung tissue upon challenge with antigen. This has also been extended to human lung tissue. Human lung tissue can be sensitized and challenged with allergen-released histamine. Prostaglandins,PGE_1 and PGE_2, in concentrations of 10^{-5} to 10^{-6} M inhibited this histamine release. With this and other associated data,it is felt that the prostaglandins exhibit

some autoregulatory role. Additional information shows that the prostaglandins have been active in human bronchial smooth muscle tissue and inactive in lung tissue. The prostaglandins are agents which exhibit a broad specrtum of physiological and pharmacological effects in a number of tissues. The prostaglandins mentioned earlier PGE_1 and PGE_2 are biosynthesized from 8,11,14-Eicosatrienoic acid and 5,8,11,14-Eicosatetraeonic acid respectively.[26] Figure 6 shows the synthesis from these agents.

Statement of the Problem

In patients suffering from allergies or allergic disorders, symptoms develop almost immediately after exposure to the offending agent. This reaction has been found to be a function of the reagenic antibodies found in the serum. As was stated earlier,the antibodies belong to the IgE class of immunoglobulins. The RAST,Radioallergosorbent Test,is a quantitative measurement of the allergen specific IgE. This test is an *in vitro* test for the determination of specific IgE antibodies. Other methods used (i.e. skin and provocation testing) seem to have several drawbacks. The allergen extracts utilized show wide variations in strength and stability,therefore a good evaluation is at best difficult. The skin test is a rather subjective measurement. This test also can be influenced by medical treatment and the physical state of an individual. Additionally,these tests are time consuming,inconvenient and sometimes hazardous to some patients.

The RIST,Radioimmunosorbent Test,while similar to the RAST is utilized for the determination of the total IgE concentration in serum and other fluids. The serum concentration of IgE is

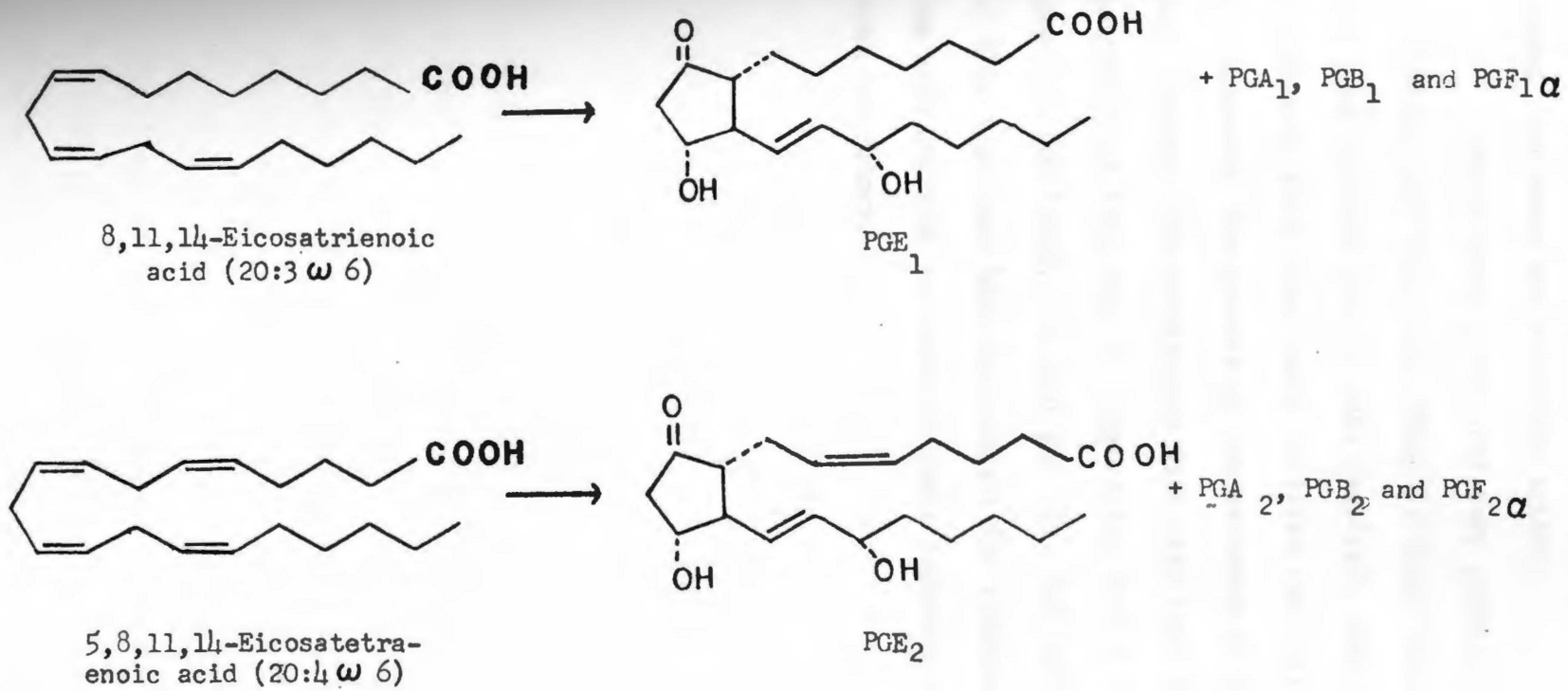


Fig.6--Prostaglandins and Prostaglandin Precursors

significantly elevated in patients with allergic disorders such as eczema, hay fever and extrinsic asthma.

While these tests look very promising, a clinical evaluation of them is most necessary. Prior to their implementation in a clinical lab, many factors such as time required, cost per test and the clinical correlation with other tests utilized for this type of evaluation must be evaluated. The purpose of the research at YSU has been to attempt such a study. The correlation with skin test data on selected allergy patients has been done in cooperation with a local allergist. The time required for tests, the cost per test and correlation with other tests of this type have been determined. The following is a detailed look at the RAST and RIST in light of their possible implementation in a clinical laboratory.

CHAPTER II

MATERIALS AND APPARATUS

Materials and Apparatus for RAST [27]

The Phadebas RAST kit procured from Pharmacia Laboratories consists of three reagent units.

The first of these reagent units is the isotope unit. This unit consists of a buffer and "Tween" solution, in dry powder form, and 0.8μg of lyophilized Anti-IgE-^{125}I with an activity of 3.5μCi at the date of manufacture. The Anti-IgE is purified to achieve a high degree of specifity and to minimize the possibility of cross reactions with other immunoglobulins. The Anti-IgE is labeled with ^{125}I at such a level as to provide high sensitivity throughout the entire shelf life of the unit. The Anti-IgE is produced from anti-serum raised in rabbits.

The next unit contains the reference reagents and contains enough reagents for a maximum of six series. This figure may vary depending upon the time between the reference series. The unit has two parts. There are 50 lyophilized reference discs and lyophilized reference sera labeled A, B, C, and D in four vials. These sera have varying degrees of reactivity to the allergens.

The third of the reagent units is the allergen discs. Each allergen of interest is available in lyophilized form in units of 25 discs per vial. Each disc is utilized for one test tube. The allergen of interest is coupled to activated cyanogen bromide paper discs by the process in Figure 7. The polysaccharide is activated

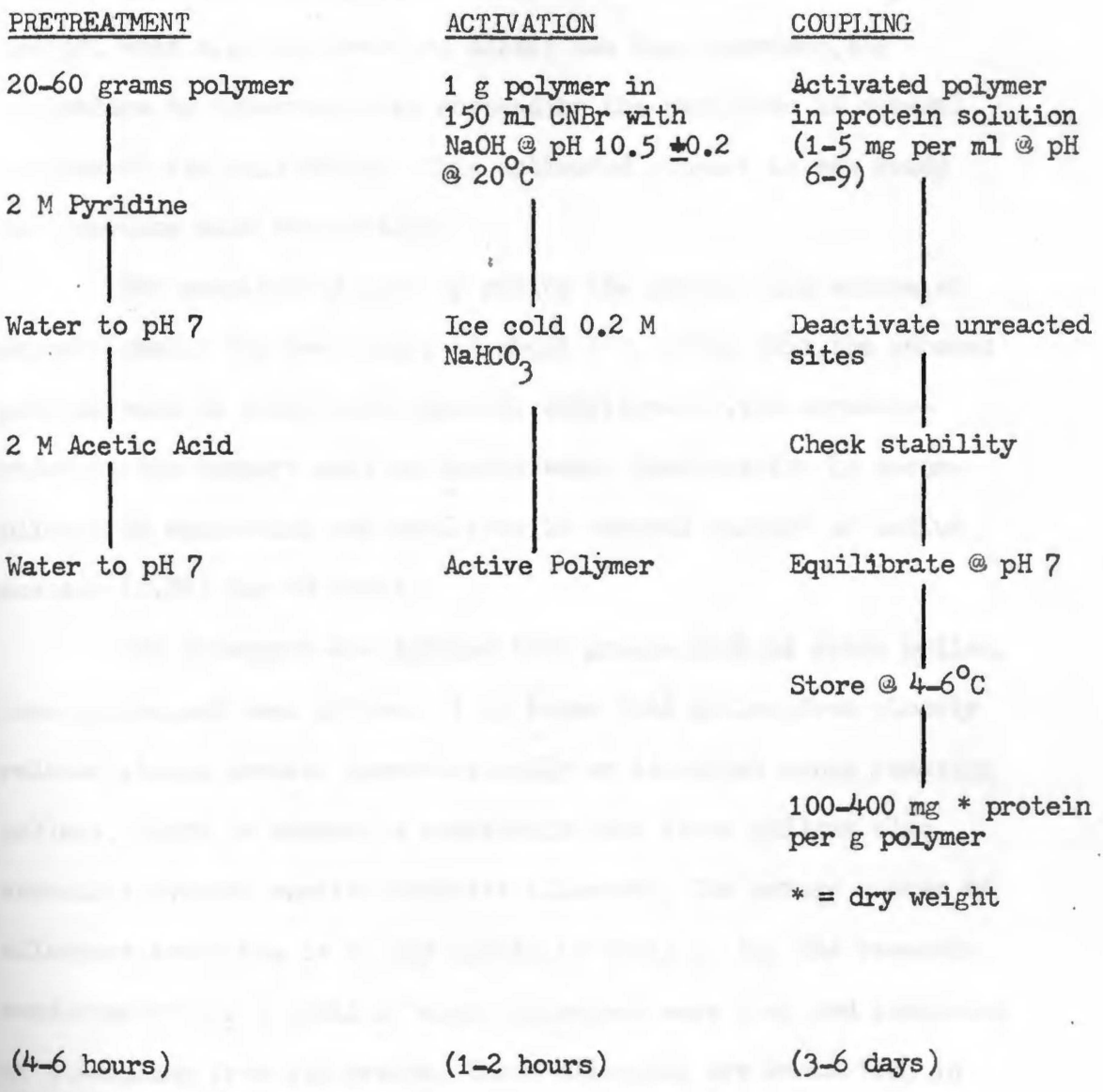


Fig.7--Scheme for Cyanogen Activation and Coupling

with CNBr (25-50 mg/ml) at room temperature at pH 10-11. In the presence of an excess of CNBr at a given pH, the degree of activation will be a function of the amount of NaOH added to adjust the pH. When a given amount of alkali has been consumed, the activation is interrupted by suspending the particles in several volumes of ice cold. $NaHCO_3$. This activated support is now ready for reaction with the protein.

The coupling is made by mixing the protein and activated support gently for 6-48 hours at about $4^{o}C$. After this, the unbound protein must be thoroughly removed. Additionally, the unreacted sites on the support must be deactivated. Deactivation is accomplished by suspending the particles in several volumes of sodium acetate (0.2M) for 48 hours.

The allergens are divided into groups such as grass pollen, tree pollen, and weed pollen. It is known that pollen from closely related plants contain immunologically or identical cross reacting pollens. There is however, a possiblity that these pollens also contain important species-specific allergens. The entire number of allergens available is 48 and listed in Table 3. For the research conducted at YSU a total of eight allergens were used and consisted of a sampling from six groups. These allergens are summarized in Table 4.

The isotope reagent unit was prepared by dissolving two components of the unit, the buffer and "Tween" solution in 400 ml. of deionized water. The resulting solution is a buffer of pH 7.4. The Anti-IgE-^{125}I was prepared by adding 5.0 ml of deionized water to the lyophilized Anti-IgE-^{125}I in the vial.

TABLE 3

RAST ALLERGEN DISCS

Grasses

- Sweet Vernal grass
- Bermuda grass
- Orchard grass
- Meadow fescue
- Perennial Rye grass
- Timothy
- Common Reed
- June grass (Kentucky Blue)

Weeds

- Common ragweed
- Western ragweed
- Giant ragweed
- False ragweed
- Wormwood (Sagebrush)
- Ox-eye daisy
- Dandelion
- English plantain
- Lamb's quarter
- Russian thistle

House Dust Mites

- Dermatophagoides Pteronyssinus
- Dermatophagoides Farinae

House Dust

- House dust (Greer Labs)
- House dust (Hollister-Stier Labs)
- House dust (Dome Labs)

Trees

- Maple
- Alder
- Birch
- Hazelnut
- Beech
- Mountain Cedar
- Oak
- Elm
- Olive
- Walnut

Epidermals

- Dog epithelium
- Cat epithelium
- Horse epithelium

Mold

- Penicillium notatum
- Cladosporium herbarum
- Aspergillus fumigatus
- Mucor racemosus
- Candida albicans
- Alterneria tenius

Foods

- Egg white
- Milk
- Codfish

TABLE 4

ALLERGENS USED FOR YSU RESEARCH

Common Name	Latin Name
Perennial Rye grass	Lolium perenne
Timothy	Phleum pratense
Common ragweed	Ambrosia elatior
Dog epithelium	
Milk	
Penicillium notatum	
Dust Mite	Dermatophagoides pteronissinus
Dust Mite	Dermatophagoides farinae

The reference discs of the reference reagent unit were prepared by adding 7.0 ml of deionized water to the lyophilized discs in the vial and these components are shaken together. This water was removed with an aspirator and 3-5 ml of the buffer solution prepared earlier was added to the vial. The reference sera A,B,C and D were reconstituted by adding 1.0 ml of deionized water to each vial. Those reference sera not used right away were dispensed into 150 μl portions and stored at -20°C for further reference series.

For each allergen of interest, 50 μl of serum is required. For 16 allergens, $\sim$1.0 ml of serum is required.

The shelf life and storage time of each reagent in the lyophilized and reconstituted form varies and can be seen in Table 5.

Materials for RIST[29]

The Phadebas RIST/IgE Test kit from Pharmacia Laboratories contains sufficient reagents for 50 single IgE determinations or 16 duplicate determinations. There are essentially four components to this kit. These components are lyophilized Anti-IgE complex, lyophilized IgE standard (400 U/ml after reconstitution), lyophilized $IgE^{125}I$ with an activity of 3.5 μCi at the date of manufacture, and buffer and "Tween" solution.

A buffer solution of pH 7.4 is prepared by adding the buffer substance to 100 ml of deionized water. The lyophilized $IgE^{125}I$ is reconstituted by adding it to 5.5 ml of deionized water. A solution of 400 U/ml IgE is prepared by the addition of 2.0 ml of deionized water to the vial. This is further diluted to make various concentration standards for the preparation of a standard curve. The final

TABLE 5

SHELF LIFE AND STORAGE FOR RAST REAGENTS

Reagent	Storage lyophilized	Storage reconstituted	Total shelf life
Anti-IgE ^{125}I	2-8°C	-20°C 2-8°C	4 months 1 week
Buffer	2-8°C	2-8°C	12 months
Tween solution	2-8°C	2-8°C	12 months
Reference discs	2-8°C	2-8°C	6 months
Reference sera	2-8°C	-20°C 2-8°C	6 months 1 week
Allergen discs	2-8°C	2-8°C	12 months

reagent consists of the Sephadex Anti-IgE complex and "Tween" suspension. The "Tween" and Anti-IgE complex are transferred into a beaker using 55 ml of the buffer solution. The beaker contains a magnetic stirring bar whose purpose is to keep the complex in solution.

In contrast to the RAST, the unknown serum is diluted ten times with buffer (0.1 ml serum + 0.9 ml buffer). The dilution is necessary since the high protein concentration of serum might influence the results. For each determination done, 0.1 ml of the diluted serum is required or about 0.1 ml of serum needed for 10 determinations.

The shelf life and storage time of each reagent varies and can be seen in Table 6.

Apparatus

Two types of test tubes were utilized for this evaluation. The type used for the RAST were 12mm by 55 mm. They were polystyrene tubes with polyethylene caps which can be procured in lots of 1000 from Pharmacia Laboratories. The RIST tubes were 12 mm diameter and 75 mm in length. They were of the same composition as the previous tubes but had no stoppers. Number 000 rubber stoppers were used when needed.

In the RIST technique, the use of a centrifuge was necessary. The one used was a Sorvall GLC-1 non-refrigerated model. Speeds of 2000-3000 rpm were used for periods of 5-7 minutes to ensure complete centrifugation of the sample. The centrifuge was used in an air conditioned lab; therefore the temperature range was 25-28^{o}C.

TABLE 6

SHELF LIFE AND STORAGE FOR RIST REAGENTS

Reconstituted Reagents	Storage Conditions
Sephadex Anti-IgE Complex Suspension	2-8°C until expiration date
Buffer/Tween Solution	2-8°C until expiration date
IgE Standard	2-8°C for 1 week or -20°C until expiration date
IgE ^{125}I Solution	2-8°C for 1 week or -20°C until expiration date

Two different scintillation counters were used during this investigation. A Baird-Atomic, Model 530 Spectrometer with the Baird-Atomic Model 810 C Well Scintillation Detector was utilized in the initial work. The well detector has a 1 and 3/4 inch diameter by two inch thick solid NaI crystal and a photomultiplier tube. With this item of equipment it was possible to dial in the background count obtained by counting with an empty tube. This equipment was manually loaded and operated and due to the large number of samples done at one time it was most necessary to utilize equipment that had the capacity for automatic counting.

As the evaluation progressed, the Packard Model 5320 Auto-Gamma Spectrometer was used. This instrument has a 300 sample capacity plus temperature controls, automatic operation and teletype output.

Both instruments had differential and integral windows, low level discriminator, window and low level reject and background subtract. The background counts obtained with both instruments were similar.

Other associated equipment included a multitip aspirator procured from Pharmacia Laboratories. This aspirator allowed the removal of solution from eight tubes simultaneously and therefore was a great time saver.

For smaller amounts of tubes or when the RIST was being done, a single tip aspirator was used. For the dispensing of various reagents in predetermined quantities a micro-macropipette was used. Two of the several types available which were used in this research were of 50 and 100 μl capacity. These tubes were equipped

with disposable tips. These kits were made by the Centaur Chemical Company.

The only other chemical used in this evaluation in addition to large amounts of deionized water was NaCl. A 0.9% (w/v) solution was required. It was prepared by adding 9 g of NaCl in one liter of water. Copious amounts were required as each sample had to be washed many times in each evaluation.

Patients

The evaluation was done on twenty-four patients from Allergy Associates of Youngstown, in collaboration with Dr. T.T. Deramo. The ages of the patients ranged from 4-65 years with an average age of 26.7 years. The sample consisted of eight males and 16 females. The average age of the female patient sample was 33.8 years while the average age of the male sample was 13.9 years. All of these people had allergic disorders of some type which ranged from hayfever to bronchial asthma. Additionally, there were seven patients who were volunteers from YSU and the Youngstown Hospital Association. These personnel were used to implement and refine the RAST/RIST technique.

Blood for the patients of Dr. Deramo was collected, centrifuged and frozen at -20°C by the Medical Sciences Laboratory of Youngstown. The serum was kept in this state until the analysis was done.

CHAPTER III

PRINCIPLES OF EXPERIMENTAL WORK AND TECHNIQUE

RAST

The allergen disc made by coupling the allergen of interest to the CNBr disc reacts with the allergen specific IgE in the patient sample. After washing away the nonspecific IgE, radioactively labeled antibodies against IgE are added, thereby forming a complex. The radioactivity of the complex is measured with a scintillation counter. The more bound radioactivity found, the more allergen specific IgE present in the sample. The count rates obtained are compared with count rates obtained from reference series run in parallel with the samples. From this data, the samples are then able to be classified according to the amount of bound radioactivity present. The relative concentration of IgE antibodies to a specific allergen is converted to a numerical scoring system ranging from 0 (no response) to 4 (maximal response). A schematic of the principle is given in Figure 8.

The technique required to accomplish the RAST procedure cannot be classified as difficult. The technique is repetitive and time consuming hence the use of items like the multi-tip aspirator and the automatic counter is most helpful.

The plastic test tubes (12 x 55 mm) are arranged in a matrix in an appropriate holder. A special holder can be purchased from Pharmacia and is most convenient. The matrix set up would be with reference

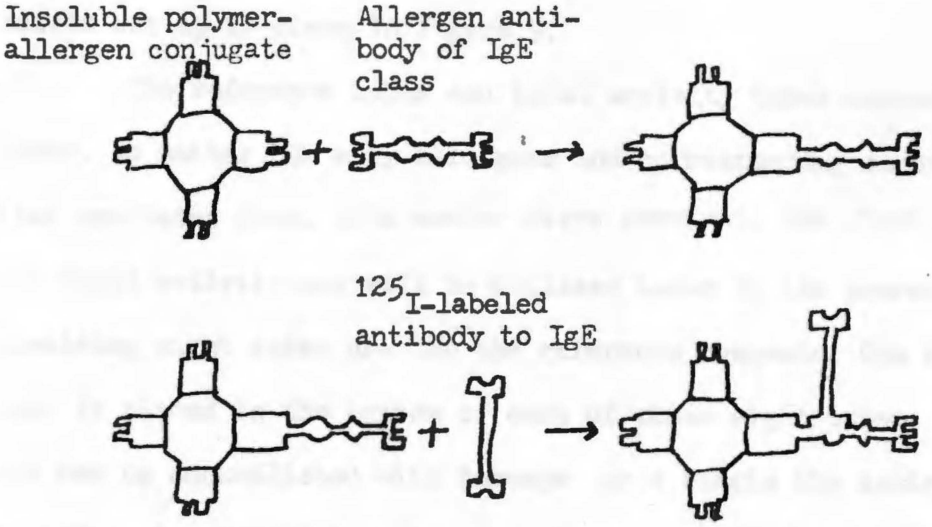


Fig.8-- Principle of RAST

reagents and the total activity tubes in one row of the matrix. The allergen discs would be placed in one direction in the remainder of the holder (e.g.right to left or up and down) while the serum sample for each patient would be in the direction at right angles to the allergen discs (e.g.right to left or up and down). An example of the matrix set up is given in Figure 9.

The reference tubes and total activity tubes account for ten tubes. No matter how many allergens and corresponding serum samples that are being done, this number stays constant. The first two tubes are total activity and will be utilized later in the procedure. The remaining eight tubes are for the reference reagents. One reference disc is placed in the bottom of each of these eight tubes. This transfer can be accomplished with forceps or a single tip aspirator. It is extremely easy to transfer more than one disc into each of the reference tubes, therefore this must be taken into consideration. Utilizing an aspirator, tends to withdraw excessive amounts of buffer. If this occurs, the buffer must be replenished.

One of the allergen discs is added to the tube for each patient. This procedure is continued until all of the necessary discs are in the appropriate tube for each patient. The same precautions apply when transferring the discs.

The next step is the pipetting of 50 μl of reference serum A into the third and fourth tubes, 50 μl of B into tubes 5 and 6, 50 μl of C into 7 and 8 and 50 μl or serum D into tubes 9 and 10. The test schedule shown in Figure 10 can be referred to for further clarification. As another precaution, the dispensing of the serum on the walls of the tubes should be avoided.

Reference Standard	A	A	B	B	C	C	D	D	TA	TA
Allergen 1	P1A1	P2A1	P3A1	P4A1	P5A1	P6A1	P7A1	P8A1	P9A1	P10A1
Allergen 2	P1A2	P2A2	P3A2	P4A2	P5A2	P6A2	P7A2	P8A2	P9A2	P10A2
Allergen 3	P1A3	P2A3	P3A3	P4A3	P5A3	P6A3	P7A3	P8A3	P9A3	P10A3
Allergen 4	P1A4	P2A4	P3A4	P4A4	P5A4	P6A4	P7A4	P8A4	P9A4	P10A4
Allergen 5	P1A5	P2A5	P3A5	P4A5	P5A5	P6A5	P7A5	P8A5	P9A5	P10A5
Allergen 6	P1A6	P2A6	P3A6	P4A6	P5A6	P6A6	P7A6	P8A6	P9A6	P10A6
Allergen 7	P1A7	P2A7	P3A7	P4A7	P5A7	P6A7	P7A7	P8A7	P9A7	P10A7

Code- P1A1 = Patient 1, Allergen 1, TA = Total Activity

Figure 9

Matrix Setup for RAST Reference Standards, Seven Allergens for Ten Patients

In each of the test tubes which contain patient allergen discs, 50 μl of the patient's serum is pipetted. The same precautions apply as in the previous paragraph. All of these tubes are covered with aluminum foil and are incubated for three hours.

After this three hour period, any liquid remaining in the tubes is removed. This is where the multi-tip aspirator is very helpful.

Into all tubes which contain discs, either reference or allergen, 2.5 ml of 0.9% saline is added. These tubes are allowed to stand for 10 minutes and the saline solution is removed with the aspirator. This procedure is repeated three times in order to remove all non-coupled IgE.

After the rinsing, 50 μl of the Anti-IgE^{125}I is dispensed into all tubes. Dispensing on the walls of the tubes must be avoided. The first two tubes called total activity contain only Anti-IgE^{125}I and are stoppered immediately. The remainder of the tubes are not stoppered. All of the tubes are covered with the aluminum foil and incubated overnight (i.e.9-12 hours).

After the incubation period, any liquid remaining is removed and all of the unstoppered tubes have their contents washed three times with 2.5 ml of 0.9% saline as before.

When this is accomplished, these remaining tubes are stoppered and bound radioactivity is then counted. In this case, a counting time of two minutes was recommended in the literature that accompanied the kit and was found to be sufficient.

The next portion will deal with the classification or results. In the procedure to be followed, the first ten tubes are counted.

As mentioned earlier, these tubes are reference tubes which have been run in duplicate. The results arrived at are mean count rates compiled by the averaging of the count rates of the two tubes. These values are used for the classification of test results. The count rates for the remainder of the tubes are filled in the appropriate columns on the result sheet. When this action is accomplished, the results can now be classified according to the schedule on Table 7. Count rates greater than those obtained for Reference A are scored as four. Those between Reference A and B are scored as three, while those in the B to C range are classified as two. When the count rate is between the values for Reference reagent C and D, one is the score. Count rates less than D are given a zero score.

TABLE 7

EXPLANATION OF RAST SCORES [30]

Score 0 = Negative, no IgE antibodies

Score 1 = Doubtful, insignificant levels of IgE antibodies

Score 2 = Weakly positive, significant but low levels of IgE antibodies

Score 3 = Moderately positive, moderate levels of IgE antibodies

Score 4 = Strongly positive, very high levels of IgE antibodies

The general precautions for the RAST and RIST procedure are similar and will be mentioned at the end of this discussion. Those precautions particular to each technique will be mentioned during the discussion of that technique. The RAST procedure is summarized in Figure 10.

Tube #	Disc	Identity	Reference or Unknown, μ l	Anti-IgE ^{125}I l
1-2	-	Total activity	-	50
3-4	Reference	Reference A	50	50
5-6	Reference	Reference B	50	50
7-8	Reference	Reference C	50	50
9-10	Reference	Reference D	50	50
11	Allergen 1	Unknown 1	50	50
12	Allergen 2	Unknown 1	50	50
13	Allergen 3	Unknown 1	50	50
14	Allergen 4	Unknown 1	50	50
15	Allergen 1	Unknown 2	50	50
16	Allergen 2	Unknown 2	50	50
17	Allergen 3	Unknown 3	50	50
18	Allergen 4	Unknown 4	50	50

Fig. 10-- RAST Test Schedule [31]

RIST

The principle of RIST or total IgE involves Anti-IgE antibodies bound to Sephadex as the solid phase. The concentration of IgE in an unknown sample is evaluated by it's capacity to compete with a fixed amount of labeled IgE for the binding sites on the Anti-IgE. This procedure is summarized in Figure 11. This competitive capacity is then compared with that of standard IgE preparations of known concentrations.

The tests are performed with the samples mixed with a given amount of iodinated IgE ($IgE^{125}I$) and an immunosorbent consisting of anti-IgE antibodies coupled to Sephadex particles (Sephadex Anti-IgE Complex). During the specified incubation period, the labeled IgE and the IgE present in the sample compete for the binding sites on the immunosorbent. The bound and free IgE are separated by simple centrifugation. The unbound IgE is removed in the supernatant and the particles of Sephadex are washed and the radioactivity bound to the particles is measured. The radioactive uptake of the immunosorbent varies inversely with the quantity of unlabeled IgE present.

The count rates obtained from standard IgE concentrations are utilized to compute values to obtain a standard curve. The count rates obtained from the unknown samples are used to compute values which are plotted against the standard curve. The relative concentrations of IgE are then read from this curve.

The technique of the RIST procedure like that of the RAST mentioned earlier is not overly difficult but could become confusing. The plastic tubes of 12 x 75 mm with the 000 rubber stoppers are used for

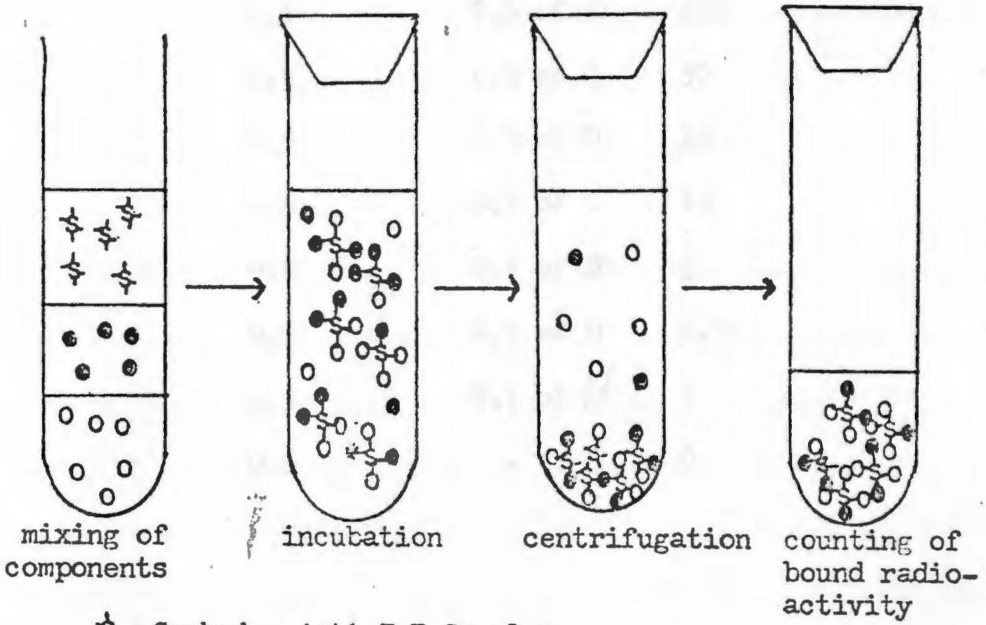
this determination. The technique calls for three "series" of test tubes called Series I, II, and III. The Series I tubes are prepared and used for the preparation of the standard curve. The Series II tubes are unknown tubes and the Series III are total activity tubes. The RIST kit will permit duplicate IgE determinations for 16 patients when the entire contents of the kit are used at one time.

The Series I tubes are labeled A to H and are done in duplicate. The labeling is very important since everything looks the same. To prepare a standard curve, the IgE standard stock solution of 400 U /ml is diluted with buffer to obtain concentrations of 1-400 U/ml. A dilution schedule is seen in Figure 12.

The concept of U(i.e. units) of IgE will be discussed in depth in later sections. At this time it will suffice to say that it is a unit of measure of IgE concentration. This concept is important in the evaluation of results. In the preparation of the standards, A to H, 0.1 ml of each standard prepared earlier by dilution is added to each tube. This is done in duplicate. For the Series II tubes, 0.1ml of each unknown serum sample is added in duplicate to each tube. The unknown serum must be diluted ten times with buffer(i.e. 0.1 ml serum + 0.9 ml buffer).

The next step involves the addition of 0.1 ml of IgE^{125}I to each of the Series I and II tubes. The same amount of IgE^{125}I is added to the Series III tubes. These tubes are stoppered and set aside. As mentioned earlier, these tubes are used to ascertain total activity and should not be washed.

The Sephadex-Anti IgE complex suspension is added in one ml. quantities to each tube in Series I and II. A word of caution



$ =Sephadex-Anti-IgE Complex

● =labeled IgE ($IgE^{125}I$)

o =IgE standard or unknown sample

Fig.11-- Principle of RIST

IgE Standard tubes	Content		Final IgE Concentration (U/ml)	Final Volume (ml)
	ml buffer	ml IgE Standard		
A	-	0.5	400	0.5
B	0.5	0.5	200	0.5
C	0.5	0.5 of B	100	0.4
α	0.5	0.5 of C	50	(0.4)
D	0.5	0.5 of α	25	0.9
β	0.9	0.1 of C	10	(0.9)
E	0.9	0.1 of α	5	1.0
F	0.9	0.1 of D	2.5	1.0
G	0.9	0.1 of β	1	1.0
H	0.5	-	0	0.5

Fig.12-- RIST Dilution Schedule

involves this suspension. It must be kept stirring continuously during the entire operation. Not only must it be kept stirring continously but the speed at which it is stirred is most important. If it is stirred too slowly the suspension will settle and concentrate. Additionally,if it is stirred too fast,the force will cause the Sephadex particles to concentrate. If this concentration does occur,the resulting data obtained when the standards and unknowns are calculated will very likely be inaccurate. This is due to the fact that the concentration of the Sephadex which occurs is uneven.

The tubes of Series I and II are stoppered and incubated overnight at room temperature. These tubes are then rotated to keep the particles suspended. Next,these tube are centrifuged at about 2000 rpm for two minutes. This serves to remove any droplets from the stoppers. The stoppers are now removed and the centrifugation is repeated. The supernatant liquid is removed with the single tip aspirator tied to the water aspirator. This removal of the liquid must be accomplished very cautiously. The liquid is removed to within about 5 mm from the bottom of the tube. If not,some of the Sephadex particles may be removed during the aspiration process and the resulting data inaccurate.

A solution of 0.9% saline previously prepared is added in two ml. quantities to each of the Series I and II tubes. The tubes are centrifuged again at 2000 rpm for two minutes. The resulting supernatant is withdrawn following the procedure in the previous paragraph. This entire procedure is repeated two more times. The tubes are then stoppered.

The tubes if Series I, II and III are counted in a gamma counter. This counting was accomplished using either the manual or the automated unit described earlier. The entire test procedure is summarized in Figure 13.

The next section will deal with the calculation of results and the expression of the data that is generated. The count rate of each of the standard tubes, Series I, is expressed as a percentage of the mean count rate of the zero tubes, tube H.

$$\frac{\text{Count Rate of the Standards}}{\text{Mean Count Rate of the Zero Tubes}} \times 100 = \% \text{ of } MCR_o \quad (3)$$

The values obtained from the calculations of the Series I tubes are plotted on lin-log paper to obtain a standard curve. The percentage values calculated are plotted versus the logarithm of the IgE concentration. A sample standard curve is seen in Figure 14. The mean count rate obtained for the zero tubes is multiplied by 0.96 to correct for serum effects. Since IgE-free serum is exceptionally hard to obtain the ratio between zero samples consisting of buffer and an IgE-free serum has been investigated and found to be 0.96. The count rate of the unknown serum is expressed as a percentage of this mean count rate, serum value.

$$\frac{\text{Count Rate of the Unknown Tube}}{\text{Mean Count Rate of the Zero Tubes} \times 0.96} \times 100 = \%\ cf\ MCR_{o\ (serum)} \quad (4)$$

These percentage figures now allow the concentration of IgE for each unknown to be read directly from the standard curve. These results obtained must then be multiplied by the dilution factor which in this case was ten. The result obtained is now the concentration of IgE. Figure 14 is a calibration curve for the RIST procedure.

Tube Identification	IgE Standards ml	Unknown ml	$IgE^{125}I$ ml	Sephadex Complex, ml
Series I (2 tubes each)				
A 400 IgE U/ml	0.1	-	0.1	1.0
B 200 IgE U/ml	0.1	-	0.1	1.0
C 100 IgE U/ml	0.1	-	0.1	1.0
D 25 IgE U/ml	0.1	-	0.1	1.0
E 5 IgE U/ml	0.1	-	0.1	1.0
F 2.5 IgE U/ml	0.1	-	0.1	1.0
G 1 IgE U/ml	0.1	-	0.1	1.0
H 0 IgE U/ml	0.1	-	0.1	1.0
Series II (2 tubes each)				
Unknown 1	-	0.1	0.1	1.0
Unknown 2	-	0.1	0.1	1.0
Unknown 3	-	0.1	0.1	1.0
Unknown 4	-	0.1	0.1	1.0
Series III (2 tubes)				
Total Activity	-	-	0.1	-

Fig. 13— Summary of RIST Procedures [32]

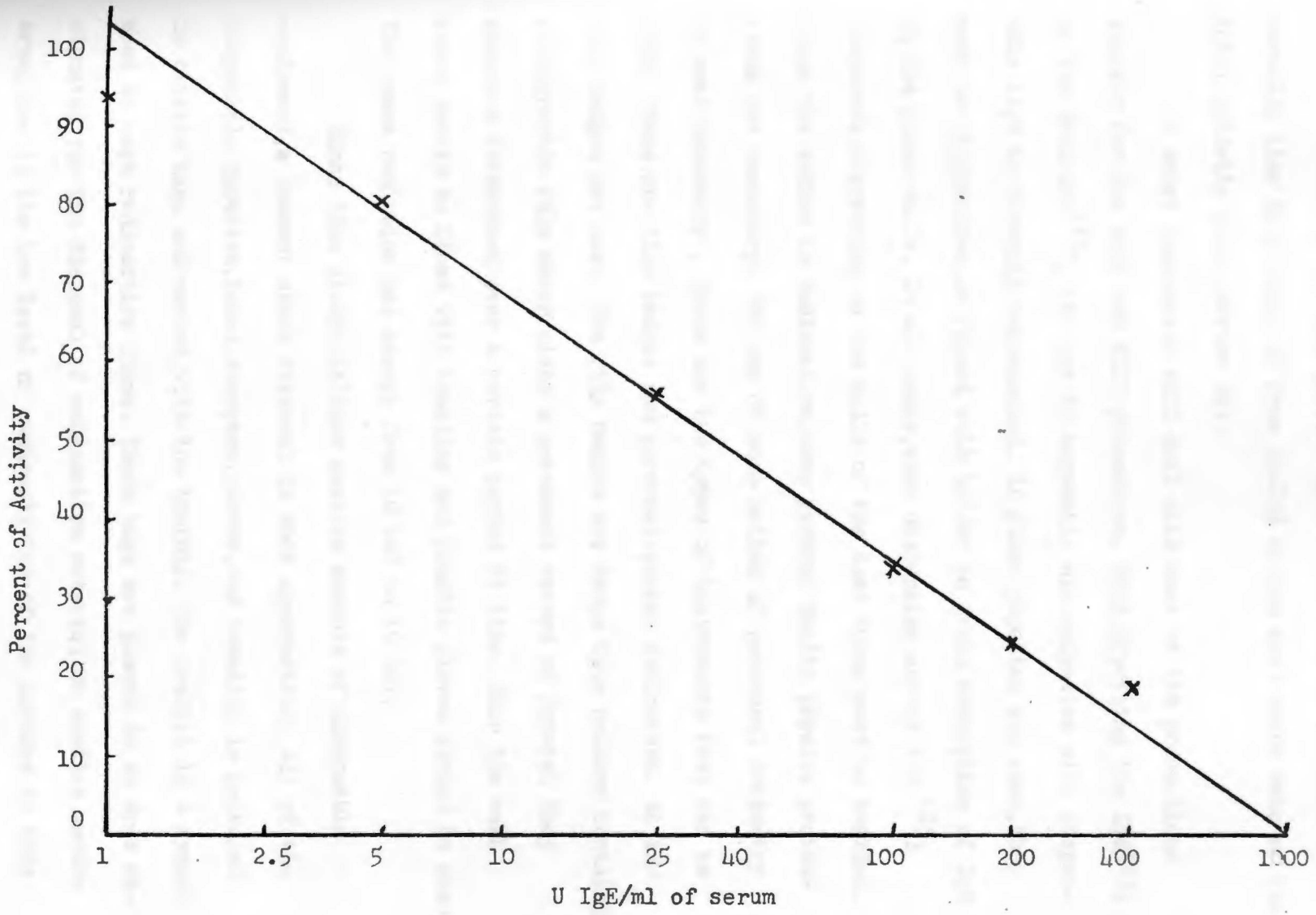


Figure 14

Calibration Curve for the RIST (IgE) Procedure

As a check of this kit, the mean count rate of the zero tubes normally lies in a range of from 10-25% of the mean count rate of the total activity tubes, Series III.

A brief discussion will deal with some of the precautions similar for the RAST and RIST procedures. When pipetting the IgE-^{125}I or the Anti-IgE-^{125}I, the use of automatic micropipettes with disposable tips is strongly recommended. If glass pipettes are used, they must be siliconized or rinsed with buffer to avoid adsorption of IgE on the glass walls. In all cases, when dispensing any of the ^{125}I compounds, dispensing on the walls of the test tubes must be avoided. Since the iodine is radioactive, some general health physics precautions are necessary. The use of some method of personnel dosimetry is most necessary . There are two types of instruments that can be used. These are film badges and personal pocket dosimeters. At YSU film badges are used. The film badges are badge type holders containing photographic film which gives a permanent record of general body exposure integrated over a certain period of time. Also the work bench should be lined with toweling and plastic gloves should be used. The gamma radiation has energy from 10 keV to 10 MeV.

Since this study utilizes massive amounts of disposable equipment, a comment about disposal is most appropriate. All of the disposable pipettes, tubes, stoppers, gloves, and toweling is packaged in plastic bags and marked with the trefoil. The trefoil is a symbol used to mark radioactive items. These bags are placed in an area designated for the disposal of radioactive material or another secure area. Due to the low level of radioactivity of the isotope in this form, there are no special precautions required by Federal Regulations for it's disposal.

CHAPTER IV

RESULTS

The results for the RAST and RIST procedure are summarized in the following tables for the four allergens used in this evaluation for the twenty four patients evaluated. The Tables 8,9,10 and 11 summarize RAST results. The RIST results are summarized in Table 12 for the seven people who were evaluated.

TABLE 8

TIMOTHY ALLERGEN VERSUS PATIENT RESPONSE

Patient	RAST Score	Skin Test Evaluation	Clinical Comments
JS	3	4+	
DM	1		no allergy symptoms but was 4+(1965)
MH	0		no pollen allergies
RY	3	4+	reactive to grasses
BM	3	4+	
DA	2		grass involvement
BB	0		
MW	4	4+	
JB	1		moderate reaction
LK	4		reactive to grasses
RH	1		no pollen allergies
JH	3		elevated eosinophilia
TY	1		
LB(1)	0		moderate reaction
CR	1		reactive two years ago
CH	2		reactive, but no symptoms
RG	3	4+	

TABLE 8
(continued)

DT	3		"correlates well"
LM	0		negative to timothy
SS	3	4+	
LE	0		bronchial asthma
LB(2)	1		wheezes around grasses
SB	3		poor timothy season
IW	4		highly reactive

TABLE 9

RAGWEED ALLERGEN VERSUS PATIENT RESPONSE

Patient	RAST Score	Skin Test Evaluation	Clinical Comments
JS	1	1+	
DM	1		no allergy symptoms since 1973
MH	1		asthma
RY	1		mild ragweed
BM	3	4+	
DA	3		ragweed involvement
BB	4		reactive to ragweed
MW	4	4+	
JB	0		moderate reactivity
LK	2		hayfever
RH	1		asthma
JC	2		hayfever
TY	3	4+	
LB(1)	0		numerous medications
CR	2	4+(1972)	
CH	4		very reactive to ragweed
RG	4	4+	bronchial asthma
DT	1		"correlates well"

TABLE 9
(continued)

LM	0		"correlates well ",negative to ragweed
SS	3	4+	hayfever
LE	2	4+	bronchial asthma
LB(2)	2		wheezes around ragweed
SB	1		
IW	1		

TABLE 10

DERMATOPHAGOIDES PTERONYSSINUS ALLERGEN VERSUS PATIENT RESPONSE

Patient	RAST Score	Skin Test Evaluation	Clinical Comments
JS	1		
DM	0		
MH	0		
RY	0		
BM	1		dust allergies
DA	0		
BB	0		
MW	0		
JB	0		
LK	0		
RH	0		
JC	2		dust allergies
TY	0		
LB(1)	0		
CR	0		
CH	0		
RG	0		
DT	0		
LM	0		
SS	2	4+	
LE	0		
LB(2)	0		
SB	0		
IW	0		

TABLE 11

DERMATOPHAGOIDES FARINAE ALLERGEN VERSUS PATIENT RESPONSE

Patient	RAST Score	Skin Test Evaluation	Clinical Comments
JS	1		
DM	0		
MH	0		
RY	0		
BM	0		
DA	0		
BB	0		
MW	0		
JB	0		
LK	1		
RH	1		
JC	2		Reactive to dust
TY	0		
LB(1)	0		
CR	0		
CH	0		
RG	0		
DT	0		
LM	0		
SS	2	4+	
LE	0		
LB(2)	0		
SB	0		
IW	0		

TABLE 12

RIST RESULTS

Patient	IgE (U/ml)
WH	72
JVN	2700
WG	600
DN	23
JT	600
CK	1100
DA	3200

CHAPTER V

DISCUSSION OF RESULTS

Introduction

This section of the study will be in four parts. The first part will deal with a correlation between RAST and RIST, skin test evaluations and clinical comments by the physician allergist. The second part will be a brief introduction and overview of possible cost figures that might be useful in considering one or both of these techniques for implementation in the clinical laboratory. The third portion will deal with recommendations to the manufacturer of the kits and to personnel considering the kits for use. The last thing that will be mentioned will be comments on the applicability of the Phadebas RAST and Phadebas Total IgE from the viewpoint of the author of this study.

Correlation Studies

A series of studies have been done on the comparison between clinical history, provocation tests, skin tests and the RAST. In all of these studies very good correlations have been obtained between the RAST and various in vivo tests. In a 1967 study, Wide found a 68% agreement between the results of 140 skin tests and the RAST.[33] A 79-82% agreement between RAST and skin prick tests with the house dust mite was found by Stenius and Wide in 1969. This study was done on patients with a history suggestive of house dust allergy.[34]

A study by Fagerberg and Wide (1970) of thirty five adults with asthma and suspected allergy to dog epithelium obtained a 97% correlation between the results of RAST and a clinical diagnosis of dog hypersensitivity as obtained from history and provocation tests.[35] Berg, Bennich and Johansson (1971) compared results from provocation tests with ninety six children, 3-15 years of age and found a 74% agreement.[36]

Further studies have shown that there was no correlation with early or late onset asthma, the age of the patient, duration, severity, and frequency of symptoms and IgE levels in the serum of patients hypersensitive to dust mites and several grass pollens. (Holford-Strevens, et al, 1970; Stenius, Wide and Seymour, 1973) [37]

In addition to the comparisons betwwen provocation testing and skin testing, there is further correlation seen between RAST and various other <u>in vitro</u> biological tests. Two such tests, both which rely on the release of histamine from sensitized human lung tissue (chopped lung and leukocyte) have been employed in conjunction with the RAST. When antibody titers to animal danders, birch and timothy pollens and codfish allergen were estimated with the chopped human lung test and compared to RAST, a highly significant correlation was obtained ($P < 0.001$)[38].

In the research conducted at YSU, a total of eight allergens were used. Of these eight, four were used for tests of the patient samples. These were ragweed, timothy, Dermatophagoides Pteronyssinus and Dermatophagoides Farinae.

A word is necessary about the procedure used in compiling data and results. The results from the RAST were filled in the

appropriate blocks on a form furnished by Pharmacia. This form is filled out in triplicate. The final copy is kept by the investigator and the other two copies forwarded to the allergist. The allergist kept one of the two remaining copies and returned the final copy to YSU with clinical comments and/or skin test results. The regression analysis was done on two of the four allergens tested. In those cases where skin test results were reported, they could be numerically compared with RAST. When clinical observations or nothing was reported, correlation was not possible. The regression analysis was done on the ragweed and timothy results. It was impossible to perform a regression analysis of the results of the dust mites as in almost all of these cases, a negative report was received.

The regression analysis was done utilizing the STAT/BASIC Option of the IBM 360/40 Computer. Due to the lack of sufficient data the analysis was performed on only two of the four allergens. Those two allergens were timothy and ragweed. The timothy allergen data utilized for the X variable was the data obtained from RAST. The Y variable data was that given to YSU by the allergist and was a result of skin test evaluation data. The value obtained for the intercept was 0.7 with a regression coefficient (i.e. slope) of 1.0. The plot for this data can be seen in Figure 15. In this case, ten X and Y values were used.

In the case of the ragweed allergen, there were eleven points for the regression analysis. The same X and Y variables were used. The data generated an intercept of 0.0 and a regression coefficient of 1.3. The plot generated is seen in Figure 16.

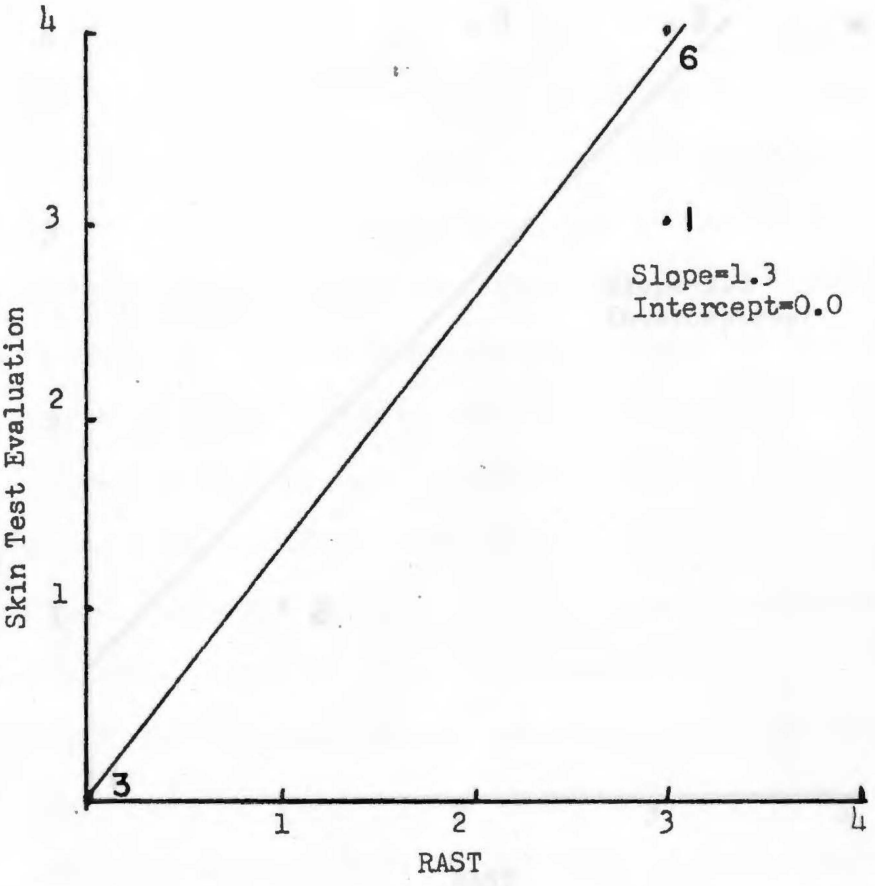


Fig.15-- Ragweed Allergen Regression Analysis Plot

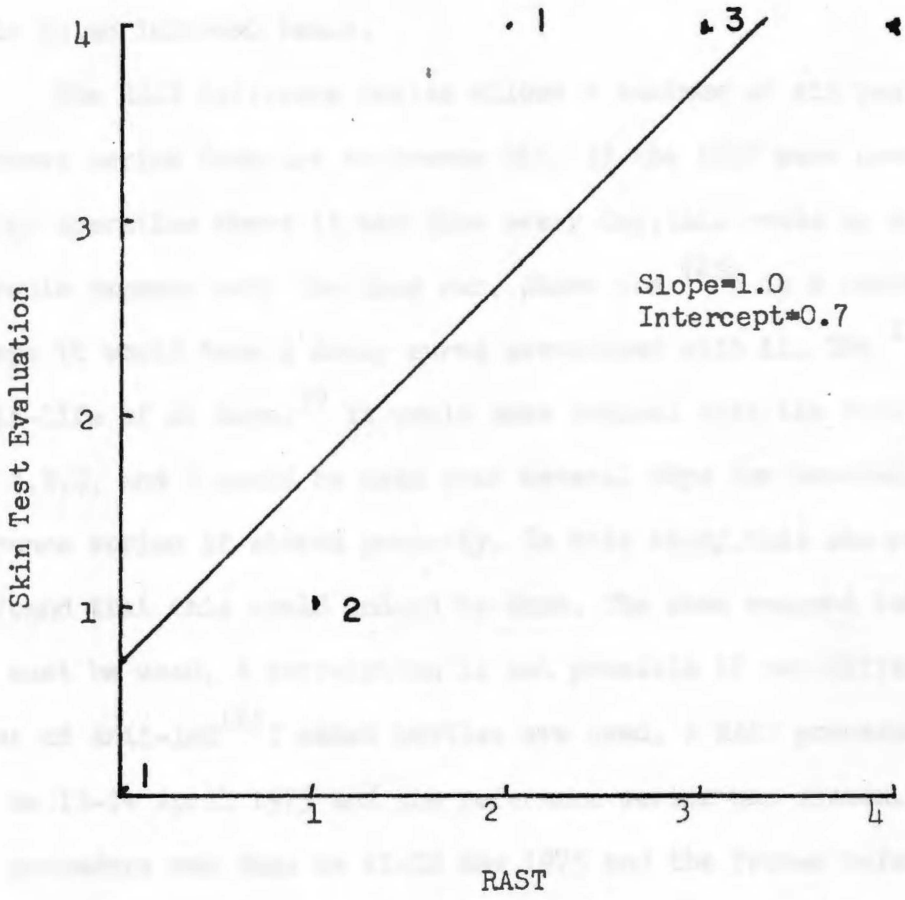


Fig.16-- Timothy Allergen Regression Analysis Plot

As mentioned in Chapter III, there were seven patients who were volunteers from YSU and YHA. While these patients were not considered in the correlation studies mentioned earlier, the results of the RAST for these people would seem to give a good correlation. This observation is based on the clinical history given by these people on an informal basis.

The RAST Reference Series allows a maximum of six possible reference series from one reference kit. If the RAST were used in a large operation where it was done every day, this could be considerable expense over the long run. Since the ^{125}I is a radioactive isotope it would have a decay curve associated with it. The ^{125}I has a half-life of 61 days.[39] It would seem logical that the reference sera A,B,C, and D could be used over several days for several reference series if stored properly. In this study, this was studied and found that this could indeed be done. The same reagent bottle code must be used. A correlation is not possible if two different series of Anti-IgE^{125}I coded bottles are used. A RAST procedure was done on 13-14 April 1975 and the reference series was frozen. Another RAST procedure was done on 21-22 May 1975 and the frozen reference from the earlier date was compared with the reference done on 21-22 May. The results are seen in Table 13.

While doing a RIST procedure, the standards A through H were done and a standard curve was obtained. The percentage values for the MCR_o of the standard tubes and unknowns are in Table 14. These percentage values for the unknown values were plotted on the standard curve and the results read out and multiplied by ten, the dilution factor. The results obtained are expressed in Units(i.e.U) of IgE

TABLE 13

COUNT RATES FOR FRESH AND FROZEN REFERENCE STANDARDS

	Total Activity	Reference A	Reference B	Reference C	Reference D
Average Counts 21-22 May (fresh)	52376	11500	5328	1559	1157
Average Counts 21-22 May (frozen on 13-14 April)	52434	12058	5026	1664	1204
Deviation	+0.11%	+4.85%	-5.66%	+6.73%	+4.06%

TABLE 14

RIST COUNT RATE AND % MCR VALUES

Standards (U IgE/ml)	Average Count Rate	% MCR (zero tube)
A (400)	507	19
B (200)	588	22
C (100)	909	34
D (25)	1496	56
E (5)	2164	81
F (2.5)	2378	89
G (1)	2458	92
H (0)	2671	100
Patient		
WH	1890	73.7
JVN	511	19.9
WG	1755	68.4
DN	2344	91.9
JT	1099	42.9
CK	813	31.7
DA	459	17.9

per ml. This value can be converted to ng per ml. In this area there is some disagreement between various literature sources. Some literature states that 1 unit of IgE equals one ng while other literature states that 1 unit of IgE equals two ng. Most of the values in this evaluation will be reported as U of IgE. A mean value of 1100 U IgE/ml was obtained for the seven patients investigated. This value seems somewhat high, but does not seem out of range. Table 15 shows a compilation of normal IgE values in several age brackets.

The serum IgE levels in non-allergic adults was found to be 250-330 ng/ml. This value was obtained by the RIST procedure, but technical improvements indicated that this value was too low. Investigations by Johansson, Bennich, and Berg have shown significant differences in serum IgE levels with several modifications of the procedure while using the same batches of anti-serum and labeled IgE.[41] The IgE level in healthy adults has been investigated by many laboratories and has been found to be in the range of 179-350 ng/ml.[42]

Patients with atopic diseases have elevated serum IgE levels. In a study of patients with extrinsic allergic asthma, a mean IgE value of 1589 ng/ml was found while patients with intrinsic non-allergic asthma have a serum IgE level of 275 ng/ml. Of the people with extrinsic asthma about 60% had a mean IgE level in excess of 700 ng/ml, corresponds to two standard deviations (S.D.) of the upper limit found in healthy adults. In asthmatic children, a mean IgE level of 563% of the normal mean for each age group was seen when allergic rhinitis was the dominating symptom. In a study of 226 unselected children with various allergic disorders, as high as 89% had an IgE level above the normal mean for their age.[43] The IgE level in an atopic

TABLE 15

NORMAL IgE VALUES [40]

Age	Mean (U/ml)	Range,2 SD (U/ml)
Newborns	1.6	0.7-3.4
6 weeks-3 months	4.4	1.1-17.2
3 months-9 months	12.9	2.6-65.2
9 months-2 years	18.4	6.4-53
2 years-4 years	27.0	7.1-103.3
4 years-6 years	42.9	7.6-242.4
6 years-10 years	55.3	7.8-391.6
10 years-20 years	86.0	12.0-618.0
20 years-70 years	71.0	10.0-506.0

individual is not constant and may fluctuate. Exposure of a sensitized patient to a relevant allergen usually leads to an increase in IgE levels. It would seem that the more allergens to which a person is allergic, the higher the serum IgE level.[43]

An interesting thing which result is that those patients subjected to hyposensitization treatment have a stimulated IgE production. After a three week period, a 40-50% increase over the pretreatment level was obtained. Also, in certain non-atopic disorders, there was a remarkable increase in IgE levels. For example, in several instances mean levels of 3520 ng/ml and 6212 ng/ml were found. These drastic levels were all due to different kinds of parasitic infections which stimulate IgE production.[44]

Another use of RIST is in the detection of IgE levels in secretions. Asthma and hayfever involve mucous membranes and IgE can be detected in secretions like mucous in the order of 500-2000 ng/ml.[45]

The values obtained by the RIST done at YSU tend to be widely scattered and the average IgE level somewhat high. Of those people who did exhibit elevated IgE levels, indications of clinical symptoms would seem to indicate that these people had some allergic reation at the time. One bit of data, dealt with one member of the test evaluation who was definitely allergic as indicated by RAST and clinical comments but who had an IgE level of 72 IgE U/ml. This while a bit unusual, doesn't seem excessive since the mean IgE level for allergic adults can be anywhere in a range from 2-1000 ng/ml depending on what literature values are used.

A research standard preparation of IgE has been made available by the WHO Reference Center for Immunoglobulins. The standard prep-

aration consists of freeze-dried pooled serum distributed in ampoules containing 10,000 arbitrary units.[46]

It has been recommended that the preparation be included in the investigation of IgE in order to make possible a comparison of values from different laboratories. The specific activity of IgE expressed in nanograms per unit,is under investigation in several laboratories.

Cost Analysis

The second portion will deal with a brief introduction into cost analysis. This will not be a rigorous examination by the discipline of cost accounting but will give material and labor costs that might be used by a clinical laboratory manager considering the RAST and /or RIST for implementation into a clinical lab. Material costs quoted are those taken from literature provided by Pharmacia Laboratories. The data on the salary for a Medical Technologist and overhead costs was procured from the YHA.

If a system such as RAST/RIST would be feasible for implementation in a clinical laboratory,a lab manager would want to compare the costs in his institution with those of another hospital. The numbers used to measure the input of resources are represented by dollars and man-hours of labor. The unit of output initially looks simple to identify. There are revenues generated from hospital systems,patients who are provided services and determinations made. Certain ratios are useful for indicators as system effectiveness. Several of these include:[47]

1. Operating Cost/Determination

2. Operating Cost/ Patient Day
3. Operating Cost/ Admission
4. Revenue/Patient Day
5. Revenue/admission
6. Determination/Labor Hour

In this evaluation, the operating cost per determination will be used. The operating cost will include cost for equipment, reagent cost, overhead, and personnel costs. Each of these components will be examined individually then combined to obtain some meaningful data. The first component of the operating cost that will be examined will be the cost for equipment. Probably the major item that is necessary is the scintillation counter. Most hospitals have this type of equipment, therefore this is not a major problem. Other necessary equipment is the 55 mm x 12 mm test tubes and the single tip aspirator. Two other items that can be classified as "nice to have" and also a great time saver are the multi-tip aspirator and a RAST test tube holder which holds eighty test tubes in an eight by ten test tube matrix. The cost of these two items is not great and in the long run, the savings in time would pay for this equipment. The equipment costs are summarized in Table 16.

The major share of the cost for this technique lies in the category of the reagent cost. These reagents include three kits: (1) Reference Unit (2) Isotope Unit (3) Allergen Unit. Another component included for completeness is the 0.9% saline solution. The cost for saline is very small when compared to the cost of the other reagents. The cost for one lb of ACS Certified NaCl according to the most recent Fisher Chemical Index is $2.25. Nine grams is needed to make one

TABLE 16

EQUIPMENT UNIT COSTS

Item	Cost	Unit Cost
1000 test tubes with caps	$34.50	$.035
Single tip aspirator	$8.50	-
Multi-tip aspirator	$8.50	-
Test Tube holder (80 test tubes)	$8.50	-

liter of 0.9% saline. This one lb would make in excess of 50 liters of this solution. About 1.5 liters are needed for each complete RAST series of 100 test tubes, therefore one lb would be sufficient for over thirty complete RAST procedures. The cost per 100 test tubes would be approximately $.07. The cost for the reference unit is $65.00. As mentioned earlier this would allow a maximum of six reference series. The cost per reference series is about $13.00. While the maximum possible reference series is six a more realistic figure would be five reference series. The isotope unit is sufficient for 100 test tubes and costs $185.00, therefore the cost per test tube is $1.85. The allergen discs come packaged in vials of 25 for $30.00 or $1.20 for each allergen disc used.

In the RIST, Total IgE, technique there is only one reagent component that has a cost of $75.00 and will allow a maximum of 16 samples to be done in duplicate. The reagent cost for each sample is $4.70. The unit cost of reagents is summarized in Table 17.

TABLE 17

UNIT COST OF REAGENTS

Item	Cost	Unit Cost
Reference Unit	$65.00/five series	$13.00
Isotope Unit	$185.00/100 test tubes	$1.85/test tube
Allergen Discs	$30.00/25 discs	$1.20/disc
Total IgE (RIST)	$75.00/16 samples	$4.70/sample

The third part of the operating cost is the overhead. This cost is a fixed cost and doesn't vary according to the number of tests performed. This cost remains constant over wide ranges of

variation in the volume of tests. This fixed cost can be represented graphically as a straight line drawn parallel to the horizontal axis. This can be seen in Figure 17. The overhead includes things such as the cost of heat, light, air conditioning and janitorial services to mention a few things. This cost can vary widely, but according to data received from the YHA about 40% of the cost of each test should be added to the costs for equipment, reagents and labor to obtain a working figure for the overhead.[48]

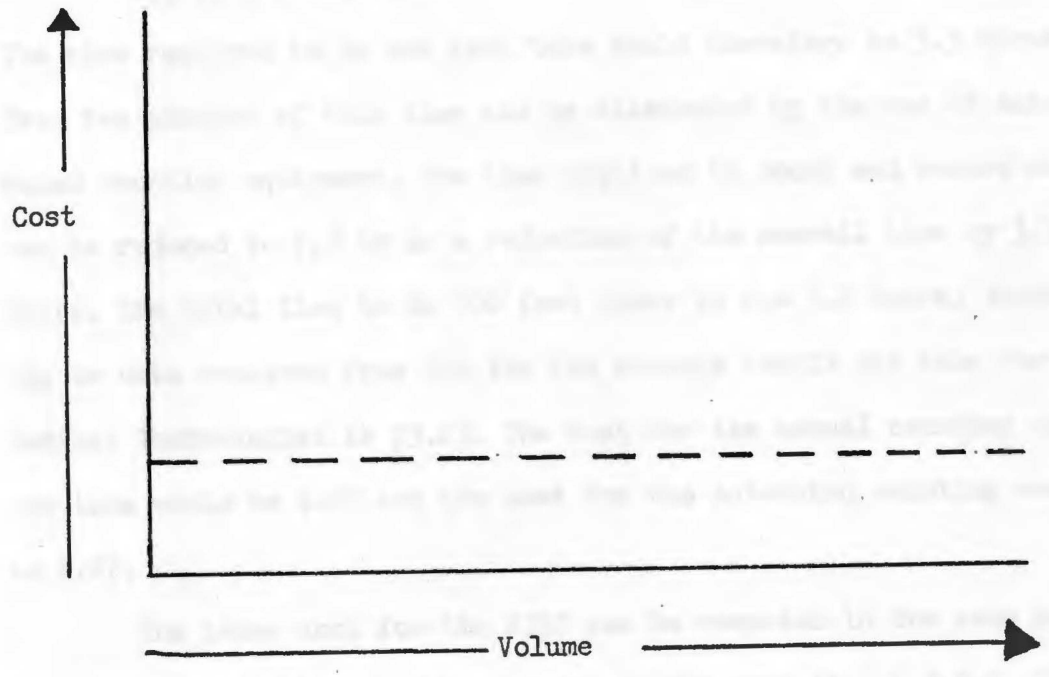


Fig.17-- Graphic Representation of Overhead Costs

Every organization includes people and the time required to perform an analysis adds the fourth part to the operating cost. This part is the labor cost. The time required to do 100 test tubes (ie. one RAST kit) can be summarized as follows:

1 hr = mix reagents

1.5 hr = set up test tubes, allergen and reference discs, add serum samples

1.5 hr = wash tube contents with 0.9% saline and activate with ^{125}I

.7 hr = wash with 0.9% saline

4.2 hr = count tubes with manual equipment and record data

8.9 hr = total time

The time required to do one test tube would therefore be 5.3 minutes. Over two minutes of this time can be eliminated by the use of automated counting equipment. The time required to count and record data can be reduced to 0.5 hr or a reduction of the overall time by 3.7 hours. The total time to do 100 test tubes is now 5.2 hours. According to data received from the YHA the average hourly pay rate for a Medical Technologist is $5.25. The cost for the manual counting of one tube would be $.47 and the cost for the automated counting would be $.27.

The labor cost for the RIST can be computed in the same manner. The time required to mix the reagents is the same (ie. 1.5 hr). The set up time is reduced to about $\frac{1}{2}$ of the RAST figure or about 0.8 hours. When the tubes are washed with 0.9% saline, this takes 0.7 hours and the time required for manual counting and data recording is the same figure as the RAST. Through the use of automated counting, a 3.7 hr time reduction can be realized. The time is summarized as

follows:

1.5 hr = mix reagents

0.8 hr = set up RIST

0.7 hr = wash with 0.9% saline

4.2 hr = manual counting and data recording

7.2 hr = total hours

In this case, the use of automated counting will cut the time required to almost ½ of the previous figure. The time required to do one tube manually is 9.4 minutes and automatically, the time is 4.8 minutes. The labor cost per test tube in the manual mode is about $.80 and in the automated mode this cost is $.40. The RAST overall cost per determination is seen in Figure 18.

The following discussion will deal only with the manual counting case as the case involving the automated counting is similar. If a technician does 50 test tubes (10 reference + 40 allergen discs), the cost per allergen is $4.35. If the total number of test tubes is increased to 200 (10 reference + 190 allergen discs), the cost per allergen becomes $3.72 and if the volume of allergens tested is increased to 990, the cost per allergen is $3.58. If these figures are compared with the data in Figure 18, a tenfold increase in volume decreases the cost per allergen tested by $.14. Another tenfold increase to a 9990 allergen figure yields a decrease of only $.03 per allergen. The cost per allergen will decrease very little after this point and will stabilize. The only thing that does occur is that the cost of the reference series tends to become less of the cost per allergen tested. Figure 19 is a graphic representation of the RAST cost per determination versus volume of tests being performed.

As mentioned earlier,a sizable portion of the cost per determination is overhead. Utilizing the 40% figure,the overhead ranges from $1.56 in the 100 test tube case to $1.42 in the 10,000 test tube case. The overall cost per allergen then ranges from $5.46 to $4.97 in the 100 and 10,000 test tube cases,respectively.

The cost analysis for the RIST technique is not quite as rigorous as for the RAST. The reagent cost per sample is $4.70. The entire RIST utilizes 50 test tubes;18 for the reference series and 32 for 16 duplicate patient samples. The labor cost for the manual method of counting is $2.51. The labor cost in the automatic mode is $1.31 per patient sample. The overhead cost ranges from $2.40 to $2.88 depending on which mode is utilized. The overall cost per patient sample in the manual mode can be calculated to be $10.10 and $8.40 in the automatic mode. A difference from the case of the RAST, is that a RIST kit contains enough reagents for 16 duplicate patient samples and if the volume of tests is increased drastically,the technician must insure that the reagents utilized have the same lot numbers. If a large volume of tests were to be accomplished special arrangements must be made with Pharmacia Laboratories to insure that this matching of lot numbers does occur.

In summary,the cost for the RAST/RIST would seem to be competitive when compared to those tests now being done in the clinical laboratory. Figures from the Youngstown Hospital Association on several test types are given in Table 18.If a large number of allergen discs were used to evaluate a patient the resulting cost might be sizable but a conservative and judicious use of the test would place it in a competitive position with other laboratory tests.

TABLE 18

REPRESENTATIVE CHARGES[48]

Test Name	Charges
Alkaline Phosphatase	$4.60
Aldolase	$15.00
Ethyl Alcohol (test for intoxication)	$22.00
NH_3 in blood	$21.00
Serum Calcium (automated)	$3.50
Total Bilirubin (automated)	$3.50
Aspirin in blood	$10.00
CPK Isoenzymes (electrophoresis)	$18.00

COST PER REFERENCE SERIES:

10 test tubes x $.035 = $.35

10 test tubes x $1.85 isotope unit/test tube = $18.50

1 series of reference reagents = $13.00

10 test tubes x $.47 test tube labor(manual) = $4.70

10 test tubes x $.27 test tube labor(automated) = $2.70

Overall Cost per Reference Series (manual) = $36.55
(automated) = $34.55

COST PER ALLERGEN DISC:

$.035(test tube) + $1.20(allergen disc) + $ 1.85(isotope unit) +

$.47(labor, manual) or $.27 (labor,automated) = $3.55 (manual)
$3.35 (automated)

COST FOR 100 TEST TUBES/ 1 RAST KIT:

1 Reference + 90 Allergen discs

Manual: $36.55 + 90(3.55) = $ 356.05 for 90 tests or $3.96/disc

Automated: $34.56 + 90(3.35) = $336.05 for 90 tests or $3.73/disc

Fig. 18-- Overall Cost Per RAST Determination

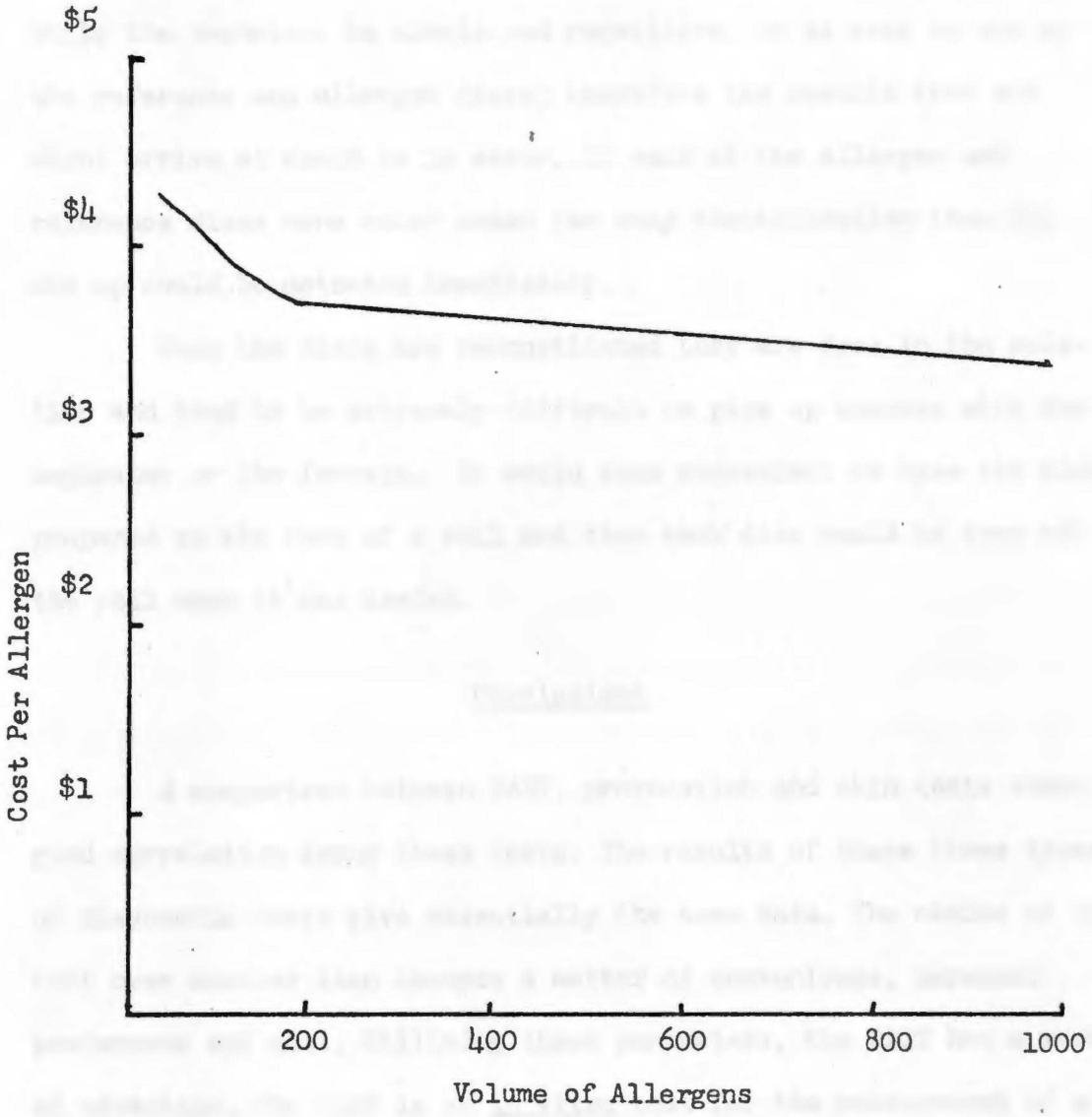


Fig.19--RAST Cost per Determination Versus Volume

Recommendations

In the course of this evaluation, several hundred tests of the RAST/RIST have been done. There are several comments that might be helpful to those people who might use or make such a kit. While the technique is simple and repetitive, it is easy to mix up the reference and allergen discs, therefore the results that one might arrive at could be in error. If each of the allergen and reference discs were color coded for easy identification then any mix up could be detected immediately.

When the discs are reconstituted they are free in the solution and tend to be extremely difficult to pick up whether with the aspirator or the forceps. It would seem convenient to have the discs prepared in the form of a roll and then each disc could be torn off the roll when it was needed.

Conclusions

A comparison between RAST, provocation and skin tests shows good correlation among these tests. The results of these three types of diagnostic tests give essentially the same data. The choice of one test over another then becomes a matter of convenience, personal preference and cost. Utilizing these parameters, the RAST has a marked advantage. The RAST is an *in vitro* test for the measurement of allergen specific IgE antibodies. The reagents for the RAST procedure are easy to prepare and the technique is simple. The RAST would lend itself easily, to high test capacity in a clinical laboratory. In an article by L. Wide, he states that the RAST is a clinically valuable tool for the diagnosis of IgE mediated allergy. It would seem very

likely that in some cases it could replace skin and provocation testing and in other cases become a valuable complement to these tests.[49]

A cost analysis for the applicability of RAST for implementation in a clinical laboratory shows that the cost is in an area to make it competitive with other techniques in the lab which utilize similar type reagents and technique.

Other areas for examination by RAST might include the examination of allergy patients and an attempt to correlate the severity of their symptoms with the class of the RAST.

The RIST is also a simple technique, but the evaluation of the data generated is not without pitfalls. The problem arises if one attempts to compare the data generated by the RIST to literature values. The values of the RIST are expressed in U IgE/ml while much of the literature is expressed in ng/ml. Some literature quotes one unit of IgE equals one nanogram while other literature states that one unit of IgE equals two nanograms. This does make the evaluation and correlation of data at best difficult. The data arrived at by YSU research is widely scattered over a wide range of values, but it is within the possible parameters for IgE concentrations.

In the cost picture, the RIST also seems competitive. Other techniques for the assay of IgE have been time consuming, complex or have needed large amounts of pure IgE. These parameters made these other techniques most unsuitable for the clinical laboratory.

Other things that would be most usefully examined by RIST are further attempts to arrive at an average IgE concentration for non-allergic adults. Also an examination of IgE levels of persons receiving large amounts of medications for their condition or those people on hyposensitization would be helpful. If these values were followed

over a period of time, one might see seasonal variations in IgE levels or decreases in IgE levels due to therapy.

In summary, the RAST and the RIST show great promise as tools for the examination of IgE levels in patients. This could be accomplished in a modern clinical lab with a minimal of additional equipment or personnel. The cost for either of these techniques is not prohibitive as to make it's implementation difficult. Both RAST and RIST also show excellent correlation when compared with other methods of diagnosis and allergy evaluation.

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