A Mathematical Analysis of Human Leukocyte Antigen Serology

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ABSTRACT

This paper presents and explores a comprehensive mathematical model for human leukocyte antigen serology, based on a mathematical formalization of the concept of specificity. This model is general enough to take into account such factors as absorption, elution, cross-reactivity, and incomplete immunization. The paper includes a presentation of the relevant immunological background and a short discussion of the underlying computational difficulty of the basic problems. Upper and lower bounds are derived for the minimal number of specificities required to explain a given set of HLA reactions, and it is shown that the numbers of antibodies and antigens involved must be no less then this minimal number of specificities. Other techniques and theorems are also presented to aid in reducing and analyzing HLA reaction matrices.

I. INTRODUCTION

Research into the problem of isolating and defining histocompatibility (HLA) antigens, antibodies and specificities is at present a subject of

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intensive immunological research. HLA antigens are found in human tissue cells and are presumed responsible for the acceptance and rejection of transplanted tissue; thus the exploration of the properties of these antigens is of practical as well as theoretical interest.

The first work known to us which gives an explicit mathematical definition and treatment of the concept of specificity is that of Nau and Woodbury [13] and Nau [12] (from which a number of results have been incorporated). The results in this paper extend the previous work and explore the relationships between antigens, antibodies, and specificities, and are useful in analyzing histocompatibility reaction matrices in order to determine the underlying antigens, antibodies, and specificities.

Section II of this paper is a presentation of relevant immunological background at a level designed to introduce these concepts to people not familiar with the field. It may be skimmed by readers having a thorough knowledge of this material, except for the last three paragraphs, which are important in justifying the mathematical model presented in Sec. III.

Section III is devoted to presenting and further justifying our mathematical model of antigen, antibody, and specificity interactions and the various resultant immunologic reactions between human tissue and antisera. Among other things, the model accounts for such factors as cross-reactivity, absorption, and elution. Furthermore, we point out that computationally, the problem of finding specificities is a very hard one, in the sense that it falls into a class of problems for which no fast algorithms are known or are ever likely to be found.

Section IV further develops the mathematical material and presents a number of heuristics to aid the researcher in analyzing histocompatibility reactions. Upper and lower bounds are derived for the minimal number of specificities required to explain a given reaction matrix, and it is shown that the numbers of antigens and antibodies involved cannot be less than this minimal number of specificities.

Section V presents an order-theoretic approach to the analysis of histocompatibility reactions. Some material from previous sections is derived from a new perspective, and additional techniques are developed. In particular, an example incorrectly analyzed by Ciftan [6] is completely analyzed.

II. IMMUNOLOGICAL DISCUSSION

This section presents some basic information about immunologic reactions and is intended for those for whom this information is novel. It then leads into a summary of what is known about the special case of the HLA antigens and their reactions. We need first to give some terms and their meanings.

An *antigen* is a substance which when introduced into an individual provokes a reaction (*immune response*) which is *specific* and which usually

has *memory*. This is to say that the response is most intense when measured against the inducing antigen and that it occurs more rapidly and is stronger when the individual encounters the antigen for a second time. Immunity is the function of the *lymphoid system*, which includes many small mononuclear cells (*lymphocytes*) which are present in small organs called lymph nodes and which also circulate in the blood. There are two major components of the immune response: one is a *cellular response*, and the other is a *humoral* or *antibody response*. While we will not here be concerned with the cellular response, as background information it may be noted that it results in the formation of *effector T* (or *thymus-dependent*) cells which can combine with the antigen, and *suppressor T cells* which regulate this response. The humoral or antibody response requires the activity of a second set of lymphoid cells (*B cells*), which in mammals are first observed in the fetal *yolk sac* and later in the *bone marrow*. The B cells include cells which excrete a special type of protein called *immunoglobulin*. There are interactions between various subclasses of B and T cells.

The set of all descendants of a single B cell is called a *clone*. A set of B cells all of which are in the same clone produce the same chemical species of immunoglobulin. Such immunoglobulin is called *monoclonal*. Monoclonal antibodies are very rarely encountered. Most frequently, several clones are stimulated and a rather heterogeneous mixture of antibodies is produced. The term *antiserum* is often used to describe a mixture of antibodies.

Immunoglobulins have as their basic structure one protein (polypeptide chain) with a molecular weight of approximately 50,000 daltons (*heavy chain*), which is bound covalently to a smaller protein of approximately 25,000 daltons (*light chain*). The N terminal ends of the heavy chain and light chain have an intimate relationship to each other and are usually held together by a disulphide bond located near the C terminal end of the light chain. The combination of light and heavy chains provides the *active site* of the immunoglobulin. It is probable that all immunoglobulins are antibodies, but the presence of an antibody can only be recognized following its combination with an antigen. Thus to some extent the definition of antigen and antibody is circular. An antigen is something that incites an antibody (among other things); an antibody is an immunoglobulin that combines with antigen in an *exothermic reaction*. The conformation of light and heavy chains varies from antibody to antibody and is largely dependent upon the linear arrangement of amino acids in certain sequences of the *variable portions* of the light and heavy chains. This variability is extreme in the first 110 amino acids from the N terminal end and cvonsists of several variable regions each up to 10 amino acids in length and separated by short constant regions. The remainder of the light chain and most of the heavy chain have a relatively constant amino acid sequence.

The chemical bonding between antiserum and antigen occurs in a relatively small site called a *ligand* of the antigen molecule. (Antisera are remarkably selective and attack preferentially antigenic structures virtually identical with those of the immunizing antigen.) There are many different classes of immunoglobulin. Most consist of dimers of 2 light and 2 heavy chains with a small amount of carbohydrate attached to the heavy chain. Some (IgA) contain an additional structure called a secretary piece, which is essential for transportation across membranes, while others (e.g., IgM) consist of pentamers of dimeric light and heavy chains. The constant portion of the immunoglobulin chain has little or no *affinity* for antigen, but has numerous biologic properties, the most noteworthy of which confer the ability to bind to certain cell membranes through *Fc receptors* on those membranes, and to activate and fix the first of a cascade of enzymes called *complement*. These properties are manifested after the active site on the antibody has combined with an antigen or when the antibody molecules are aggregated by some physical treatment.

An antibody response is usually induced by injecting antigen into a suitable *recipient*. Sometimes a large volume is required, as for example in the production of sera for commercial applications, in which case a large animal such as a horse or goat acts as a recipient. For experimental purposes laboratory animals (including rabbits, rats, and even mice) are used, each species having peculiar advantages and sometimes disadvantages.

The antigen can vary greatly. The majority are chemical compounds. Common examples are tetanus toxoid—a formalin-treated extract of the tetanus organism—and bovine serum albumin. Some antigens are relatively simple, consisting for example of a synthetic random copolymer of several amino acids. In general an antibody response develops best when the antigen exceeds a minimum molecular weight (e.g., 10,000 daltons), is chemically heterogeneous, and differs markedly in species of origin from the recipient. Bovine serum albumin, an example cited above, is a strong immunogen for a rabbit but would be less good for a sheep and very poor for another bovine. Antigens are considered to require two components: one is called the *carrier* and is of necessity macromolecular; the second is usually small, sometimes consisting of as few as 5 amino acids, and constitutes the ligand or *immunodominant site*. Not infrequently a single short sequence of a large molecule may serve as the ligand, the remainder of the same molecule serving as the carrier. It is easy to see then that several amino acid sequences or carbohydrate side chains of a single antigenic substance can function as antigens. A recipient may respond to one or several of these ligands or may fail to respond to any, the difference in responsiveness often being determined by genetic factors.

Genetic factors can be of several classes. One class of genes controls the capacity to respond immunologically. These are called immune response genes, and every species appears to possess many such genes. A second class of genes determines *antigen structure*. Thus in immunization using antigens from a donor animal or human, if the species of origin of the antigen and the species or origin of the antibody producer shared a *structural gene* for the same amino acid sequence, then that protein or peptide would not function as an antigen. It is generally the case that no individual mounts an antibody response against a structure which is exposed on the surfaces (*cell-surface antigen*) of the tissues of his own body. This phenomenon was named by Paul Ehrlich *horror autotoxicus*, and indeed, when an individual does recognize and respond to antigens found in its own body, *autoimmunity* and *autoimmune disease* are likely to develop.

We now turn to the special case of antibodies made against cell-surface determinants of higher animals. The following statements are based on observations of all vertebrate animals studied, but have special reference to man. There are obvious differences between species, and there are also differences within a single species, which may be gross (as between a poodle and a bulldog) or may be so slight as to require laboratory tests to detect them. The immunologist and biochemist can recognize many differences in structure within a species. One such set of structures is found on the surface membrane of the cells of the body. These antigenic surface features are called *alloantigens*. Best known are the alloantigens of the A and B blood groups, the ligands or determinants of which are carbohydrates, and the Rh antigens, which are proteins. *Alloimmunization* by alloantigens (immunization within a species) induces *alloantibodies*. Alloantibodies will react against cells of the *immunizing donor*; they will also react with cells of other individuals of the same species having the same genetic determinants and therefore the same *antigenic markers*. Interestingly, they will occasionally react with similar antigens of other members of the species. Since this reaction is not one between antibody and its *homologous* antigen, it is usually qualitatively and quantitatively less strong, and is usually referred to as *cross-reaction*. Cross reactivity has long been studied in other branches of immunology. Antibodies to the ligand (or *haptens*) dinitrobenzene cross react very strongly with trinitrobenzene, less strongly with dinitrotoluene, and only weakly with trinitrotoluene. The strength of binding, called *affinity*, can be measured quantitatively with great precision and gives a measure of the intensity of cross-reactivity between such simple ligands.

The antigen-antibody reactions against lymphocytes or tissue cells are much more complex, and precise measurements are difficult. Immune responses in the human against tissues or cells from other humans would in fact have received only cursory attention if it were not for the fact that such

responses are important in platelet, white-cell, and bone-marrow transfusion, in the transplantation of tissues and organs, in the relationship between mother and fetus, and in the association between certain genetic markers of leukocytes and a variety of diseases. Because of these practical considerations many laboratories have investigated *human leukocyte alloantigens*, have generated considerable volumes of data and are in need of help in the interpretation of these data. Although there are many antigens on the surface of tissue cells, those of one particular genetic system are paramount in their biologic significance. This system is called *HLA*. It has been most extensively studied on blood lymphocytes, but it is also present on other elements in the blood—especially blood *platelets*, which are particles, smaller than lymphocytes, essential for the coagulation of blood. The HLA antigens are also found on the cells of the kidney, heart, skin, liver, and other organs and tissues of the body.

The name HLA was originally given to a series of glycoproteins of molecular weight approximately 55,000 daltons with some superficial resemblance to the immunoglobulins in that the basic structure is a glycoprotein which is associated with a smaller peptide. As with the immunoglobulins, the heterogeneity of HLA resides in its amino acid sequence. However, there are many important differences: the HLA molecule has a hydrophobic portion which is inserted into the cell membrane, variations appear to involve only isolated amino acids rather than longer sequences, and the "light chain" is a well-known protein called B_2-microglobulin. B_2-microglobulin has a molecular weight of 11,000 daltons and shows no variability between individuals.

At first only one series of HLA antigens was recognized. It was soon realized that there were two and later at least three different series each coded for by a separate genetic locus. The three known loci are called *HLA-A*, *HLA-B*, and *HLA-C*. HLA-A, B, and C series antigens are detected by the cytotoxicity test, which will be explained later. A fourth locus, *HLA-D*, is identified by a completely different procedure called the mixed lymphocyte reaction (MLR). Each HLA locus exists in several variant forms. There seems to be at least 20 variants or *alleles* at the A locus, an equal number at the B locus, at least 5 at the C locus, and at least 8 at the D locus. To further complicate the system, at least two more genetic loci are known to exist within the HLA system. These code for a different type of protein which is not associated with B_2-microglobulin and which is found in abundance on B lymphocytes but not present on platelets. (Its existence on T cells is disputed.) One of the B-cell loci is located at or near HLA-D.

The genetic complex HLA, with its minimum of 6 genetic loci, is located on the 6th chromosome. Since the 6th chromosome is an autosome, each individual has information coding for 12 antigenic molecules. To simplify this discussion we will not further be concerned with products of those

genes expressed only on B cells, nor with HLA-C or HLA-D. Emphasis will be on the products of HLA-A and HLA-B; thus individuals may be thought of as having only 4 HLA antigens. The mathematician may bear in mind the problems likely to be encountered when the additional 4 or more loci are to be analyzed.

Most of the antisera used in testing for HLA antigens come from women who have borne children, and result from immunization of the mother against the fetus. Since the fetus usually differs from the mother by one HLA-A and one HLA-B antigen, the majority of such sera contain two distinct antibodies. These can be separated by manipulations called *absorption* and *elution*. To absorb, the serum is mixed with platelets known to carry only one of the relevant antigens. The corresponding antibody combines with the absorbing platelet, which is then mechanically removed, carrying the bound antibody with it. (The residual fluid is called *absorbed antibody*—rather a misnomer.) The platelets are then treated with an acid solution, releasing the bound antibody. Again mechanical removal yields a clear solution which contains the second antibody. As an example, suppose a given serum contains anti-HLA-A3 and anti-HLA-B7. Then absorption with platelets from a donor who had A3 but not B7 would remove anti-A3 and leave anti-B7 in solution. The anti-B7 solution would be called "absorbed anti-B7". Acid treatment of the platelets would release the anti-A3 antibody; this would be called "anti-A3 eluate". *Differential elution* removes weakly bound antibodies by weak acidification and progressively more strongly bound antibodies by progressively stronger acidification, thus producing several different eluted *fractions*. If every sample of cells which removes any reactivity from a serum removes all reactivity, then the serum is said to be *operationally monospecific*.

While the binding of antibody with antigen is generally visualized as a "lock and key"-type interaction, it is not immediately apparent how this applies to a large molecule such as the HLA glycoprotein, which is far too large to be included in the active site of an antibody. The globular structure of immunoglobulin can be demonstrated by x-ray diffraction and structural analysis based on amino acid sequences. These studies show a hollow core in which the ligand fits. At present, immunologists do not know the structure of the ligand, and it may be several years before this information is available. In the meantime, the identity of antigenic specificities and the nature of cross reactivity must be deduced. For a variety of reasons it is unlikely that carbohydrate is responsible for HLA specificity, and the protein sequence is believed to be important. The ligand could be a pinched-up loop; it could be an immunodominant loop, in which a linear sequence of amino acids would determine conformation and charge; or it could involve the coincidence of several separated amino acids brought together solely by the folding of the molecule. Thus cross reactivity could

occur if two individuals had a nearly identical amino acid sequence in the immunodominant loop, if the loop were shifted slightly because of differences somewhere else on the molecule, or if there were slight differences in folding. While slight differences in composition or form of the antigen can make a difference in the antigen, different individuals probably "see" the same antigen in a slightly different manner, and it is very rare for two anti-HLA antibodies of the same general specificity to react in an identical manner when tested on a large panel of donors. Differences in the number and spacing of antigenic molecules or in the ionic charge of adjacent molecules may also contribute to differences in binding. Finally, it was clearly shown by Landsteiner that antibodies could often react with two molecules of radically different composition. This abberation can now be largely explained on the basis of similarities in three-dimensional structure, but it does offer a warning that a knowledge of the amino acid sequences of two cross-reactive antigens may not immediately explain cross reactivity.

Experimental determination of the presence of antigens is usually done by an indirect reaction called the cytotoxicity test. As stated earlier, the combination of antibody with antigen is exothermic and also results in changes which, among other things, permit the fixation and activation of complement. The exothermic reaction is a primary one, and in an ideal situation a measure of the heat of combination would give much information about the reaction of antibody and its cellular target. In practice this is inconvenient, and the immunologist is forced to the fixation of complement. This is at best a secondary event, and its measurement is subject to several sources of error. The test itself consists of mixing lymphoid cells from the blood of an individual with a small quantity of antibody. After incubation a source of complement, usually from rabbit serum, is added and, after a further incubation activation of complement, is detected by adding a dye. Dead cells are permeable to the dye and become stained. Live cells are impermeable and remain refractile and unstained. There are many variations in technique and in the indicator used, but the method described is most widespread in its acceptance, and the results of different procedures are highly correlated.

The antibody used comes from one of two main sources: women who have borne children (*multip sera*), as mentioned earlier, and subjects who have been injected with lymphocytes from another individual (*planned immunization*). Parous women frequently become sensitized by the passage of cells across the placenta during successive pregnancies, so the specificity of multip serum depends upon the antigenic relationship between mother and child and is somewhat random. Planned immunization permits the selection of donors and recipients who differ only at the HLA characteristic selected. However, both types of antisera can contain a rich variety of antibodies, some of which are not detectable by cytotoxicity testing, and the

existence of many of which is therefore not suspected. An antibody which does not fix complement can be present in a serum containing a complement-fixing antibody and can compete with it. If the two antibodies had identical reactivities the effect of the mixture would be to weaken the complement-dependent cytotoxicity reaction. If the two antibodies in the same serum had different reactivities, the presence of the second antibody would be unsuspected and its effects might only be inferred by the failure of the serum to lyse cells carrying the relevant specificity. Alternatively, the interaction of two antibodies in a serum with a lymphocyte might cause cell death where each antibody acting alone might not.

An important facet of HLA testing is that of the reaction strength, measured in terms of the portion of cells killed by a given antiserum [4]. If samples of lymphoid cells from several different individuals are each tested against the same set of antisera, the results may be tabulated in the form of a matrix in which each entry is a measure of the strength of reaction between a particular sample of cells and a particular sample of sera. The situation is complicated by the fact that the strength of each reaction depends on the presence or absence of cross reactivity or of more than one reacting antigen-antibody pair; the strength, age, and concentration of the antiserum; preferential reactivity against a subpopulation of cells; and other factors. In addition, the measured reaction strength may vary with the subjective responses of the observer, and some of the data may be missing or in error.

A model which adequately deals with the problem of varying reaction strengths is one which notes that the population of cells has subpopulations (e.g. the subpopulation of B cells in the population of small lymphocytes which includes B cells and T cells) which have been found to have specificity contents which are subsets of the total [7]. By physically separating the various subpopulations, we can reduce the problem to one in which either virtually all cells are killed or else virtually none are. This allows us to simplify the problem by partitioning the set of possible reaction strengths into two values: reaction, denoted by 1, and no reaction, denoted by 0. Thus, we may represent the data obtained from such an experiment by a Boolean incidence matrix in which each row represents the reactions of some cell to each of the sera, and each column represents the reactions of some serum to each of the cells.

From the outset it has been emphasized that the antigen is detected only through the antibody and that the reactivity of the antibody is known only through some function of its reaction with antigen. Thus in HLA serology the definition of specificities is entirely arbitrary. In the early evolution of knowledge of the system van Rood determined the reactions of 64 unselected multip sera against cells from 100 unrelated individuals. He compared the reactions serum by serum and could thus allocate certain anti-

genic specificities to cells from some donors and serum specificities to antisera reacting against those cells. This practice has been followed by all subsequent investigators.

III. THE MATHEMATICAL MODEL

At present, immunologists do not have chemical tools at their disposal to isolate individual antigens, antibodies, and specificities. Thus, the identification of these entities must be done by studying reaction matrices. Given a set of cells, a set of sera, and a tabulation of the reactions between them, it is the task of the tissue typer to find assignments of sets of antigens to the cells, sets of antibodies to the sera, and specificities to the antigens and antibodies which adequately explain the tabulated reactions.

One essential property of an adequate explanation is that a serum cannot attack a cell unless at least one of the antibodies in the serum has a specificity in common with one of the antigens in the cell. We may formulate this mathematically as follows. Let C be a set of cells, D be a set of antisera, and the relation $R \subset C \times D$ be the relation defined by $c R d$ iff the serum d reacts with cell c. Then we must find (1) a set A of antigens, (2) a set B of antibodies, (3) a relation $U \subset A \times B$ defined by $a U b$ iff the antibody b attacks the antigen a, (4) a map $g: C \to 2^A$ [$g(c)$ is taken to be the set of antigens contained in the cell c], and (5) a map $h: D \to 2^B$ [$h(d)$ is taken to be the set of antibodies contained in the serum d] such that if $c \in C$ and $d \in D$, then $c R d$ iff there exist $a \in g(c)$ and $b \in h(d)$ such that $a U b$. In such a case we say that U, g, and h *explain* R. Note that we are using the usual mathematical notation 2^A to represent the set of all subsets of A, as well as the notation $c R d$ to denote $(c,d) \in R$. We shall also use $|A|$ to represent the cardinality of A. Note that g and h above may equivalently be thought of as relations $g \subset C \times A$ and $h \subset D \times B$.

NOTATION 3.1

Generally, throughout the rest of this paper, whenever we refer to variables A, B, C, D, R, U, g, and h, they will be as defined above. Note that A, B, C, and D are finite sets.

If $X = \{x_1, \ldots, x_m\}$ and $Y = \{y_1, \ldots, y_n\}$ are finite sets and $P \subset X \times Y$ is a relation, then the *Boolean incidence matrix* for P is the matrix $[m_{ij}]$, where $m_{ij} = 1$ if $x_i P y_j$ holds and $m_{ij} = 0$ otherwise. Since a binary relation and its Boolean incidence matrix completely determine each other, we shall use these terms synonymously herein; thus a row of P shall be a row of the Boolean incidence matrix for P, etc. Note that the Boolean incidence matrices for R and U above are, respectively, the cell-serum reaction matrix for C and D and the antigen-antibody reaction matrix for A and B. Thus we shall refer to R and U, respectively, as the cell-serum reaction relation (or matrix) and the antigen-antibody reaction relation (or matrix).

If we are given sets of cells and antisera, and if we are told what serological specificities are possessed by each cell and serum, the reaction matrix between the cells and sera is completely determined from this data. The problem which faces the tissue typer is the inverse one of discovering the serological specificities given the reaction matrix. There are in general a number of different possible ways that specification could be assigned to give a particular reaction matrix; the problem is to find that particular assignment of specificities which actually is responsible. Those doing this research will generally already have some idea which cells or sera possess which specificities, but some of these assignments may be in error, and the properties of some cells or sera will be unknown. Thus the researchers may provisionally hypothesize that certain antigens and antibodies contain certain specificities, and then test them against additional cells and sera (e.g. by running absorption and elution experiments) to see if this hypothesis remains consistent with the new data. More frequently, they may run across violations of assumptions in the course of their work. For example, HLA specificities which had been worked out for Caucasian populations required considerable revision when other groups were tested [14].

It is easy to see that the set of all reactions due to a single serological specificity is the Cartesian product of the set of all cells containing the specificity and the set of all antisera containing the specificity. Thus, when analyzing a reaction matrix, it is natural to suspect that blocks of "1" elements in the matrix which are expressible as Cartesian products may be caused by the same serological specificity. This motivates the following definition:

DEFINITION 3.2

Let X and Y be sets and $P \subset X \times Y$ be a relation. Then $S \subset X \times Y$ is a *P-specificity* if (1) $S \subset P$ and (2) there are sets $X' \subset X$ and $Y' \subset Y$ such that $S = X' \times Y'$.

If R is as defined previously, then every serological specificity for R is an R-specificity, but there may be many R-specificities which are not serological specificities. When it is necessary to distinguish them from serological specificities, we shall refer to specificities as defined above as *formal specificities*.

In analyzing a reaction matrix, we would not consider our analysis complete unless every reaction could be explained by some serological specificity. Since we shall be trying to identify serological specificities by looking at formal specificities, we are led to make the following definition.

DEFINITION 3.3

If X and Y are sets and $P \subset X \times Y$, then a *P-specificity cover* (or *P-cover*) is a class $\mathbf{S} = \{S_1, \ldots, S_k\}$ such that each $S_i \in \mathbf{S}$ is a P-specificity and $S_1 \cup S_2 \cup \cdots \cup S_k = P$.

This notation is different from that used in [12], but it is conceptually equivalent and syntactically simpler.

If U is an antibody-antigen reaction relation which explains a cell-serum reaction relation R, then every U-specificity gives rise to an R-specificity, as shown in Theorem 3.4 below. Similarly, every U-cover gives rise to an R-cover, as shown in Corollary 3.5. Thus, in particular, if we can find a set of serological specificities forming a U-cover, we will have completely accounted for all the reactions in R. The proofs of these two results are very simple, but we shall first need the following mathematical notation, which is fairly standard.

NOTATION

If $P \subset X \times Y$ is a relation, then the *domain* of P ($\mathrm{Dom}\,P$) is the set $\{x \in X | xPy \text{ for some } y \in Y\}$, and the *range* of P ($\mathrm{Ran}\,P$) is the set $\{y \in Y | xPy \text{ for some } x \in X\}$.

Thus if S is a serological specificity, $\mathrm{Dom}\,S$ and $\mathrm{Ran}\,S$ are the sets of all cells and sera, respectively, which possess the specificity S.

THEOREM 3.4

Let A, B, C, D, R, U, g, and h be given, and let S be a U-specificity. Let $T = \{c \in C | g(c) \cap \mathrm{Dom}\,S \neq \varnothing\} \times \{d \in D | h(d) \cap \mathrm{Ran}\,S \neq \varnothing\}$. Then T is an R-specificity. (We say that T is induced *by S.)*

Proof. T is obviously a Cartesian product. Thus we need only show $T \subset R$. Suppose cTd, there are a $\in g(c)$ and $b \in h(d)$ such that aSb. But then aUb, so cRd. ■

COROLLARY 3.5

In the above, let $\mathbf{S} = \{S_1, \ldots, S_k\}$ be a U-cover. Then $\mathbf{T} = \{T_1, \ldots, T_k\}$ is an R-cover, where for each i, T_i is the specificity induced by S_i. (We say that $\mathbf{T}$ is induced *by* $\mathbf{S}$.)

Proof. By Theorem 3.4, each T_i is an R-specificity. If cRd, then there are $a \in g(c)$ and $b \in h(d)$ such that aUb. But then aS_ib for some S_i, whence cT_id. Thus $T_1 \cup T_2 \cup \cdots \cup T_k = R$. ■

Given A, B, C, D, R, U, g, and h, not every R-cover is induced by a U-cover, for the reason that not every R-specificity is induced by a U-specificity. However, as shown in Theorem 3.8, if we are given only C, D, and R, then for every R-cover there exist *some* A, B, U, g, and h for which there is a U-cover inducing the R-cover. In analyzing a cell-serum reaction matrix R, we are often more interested in the serological specificities causing the reactions than in the particular antigens and antibodies containing these specificities. This theorem allows us simply to hypothesize R-

specificities to be serological specificities, with the confidence that if we find a specificity cover in such a way, it will be possible to postulate antigen and antibody content for the cells and sera and serological specificity content for the antigens and antibodies consistent with this hypothesis.

DEFINITION 3.6

Let X and Y be sets, and $P \subset X \times Y$ be a relation; let $x \in X$, $y \in Y$, $X' \subset X$, and $Y' \subset Y$. Then

(1) $X'P = \{y \in Y | xPy$ for some $x \in X'\}$,
(2) $PY' = \{x \in X | xPy$ for some $y \in Y'\}$,
(3) $xP = \{x\}P$, and
(4) $Py = P\{y\}$.

Note that if $X = \{x_1, \ldots, x_m\}$, then the elements of the set x_iP correspond to the "1" elements of the ith row of P. Thus, in keeping with our identification of P with its Boolean incidence matrix, we shall refer to x_iP as the ith row of P. Thus if $X' \subset X$, then $X'P$ is the union of the rows of P corresponding to the elements of X'. For example, if X were a set of cells and P were a reaction relation, then XP would consist of all antisera in the set Y which would attack some cell in X.

Similar comments hold for the columns of P.

DEFINITION 3.7

If $f: X \to Y$ is a function and $X' \subset X$, then $f(X') = \{y \in Y | y = f(x)$ for some $x \in X'\}$.

Note that $X'P \cap Y' \neq \varnothing$ iff $X' \cap PY' \neq \varnothing$ iff there are $x \in X'$ and $y \in Y'$ such that xPy. Thus in our definition of R and U at the beginning of this section, we could have replaced "cRd iff there exist $a \in A$ and $b \in B$ such that aUb" by "cRd iff $g(c)U \cap h(d) \neq \varnothing$" or by "$cRd$ iff $g(c) \cap Uh(d) \neq \varnothing$".

THEOREM 3.8

If C, D, and R are given and $\mathbf{T} = \{T_1, \ldots, T_k\}$ is an R-cover, then there exist A, B, U, g, and h together with a U-cover $\mathbf{S} = \{S_1, \ldots, S_k\}$ such that U explains R and S_i induces T_i for each i.

Proof. Let $A = \{a_1, \ldots, a_k\}$ and $B = \{b_1, \ldots, b_k\}$ be sets of cardinality k, and put $(a_i, b_j) \in U$ iff $i = j$. For $c \in C$ and $d \in D$, put $g(c) = \{a_i | c \in \mathrm{Dom}\, T_i\}$ and $h(d) = \{b_i | d \in \mathrm{Ran}\, T_i\}$. Put $S_i = \{(a_i, b_i)\}$ for each i. Obviously $\mathbf{S} = \{S_1, \ldots, S_k\}$ is a U-cover. Suppose cRd. Then cT_id for some i, so $a_i \in g(c)$ and $b_i \in h(d)$, but a_iUb_i. Conversely, if there are $a_i \in g(c)$ and $b_j \in h(d)$ such that a_iUb_j, then $i = j$, so cT_id, so cRd. Thus U explains R. By an almost identical argument, each S_i induces T_i. ■

Of the other mathematical models of the HLA system known to us, most (e.g. [4, 6, 16]) have assumed either total immunization, absence of cross-reactivity, or both. Our model requires neither of these restrictions. Absence of total immunization is obviously no problem herein, for nowhere do we make any assumptions about the degree to which any individuals have been immunized against any others. However, to show that our model deals adequately with cross-reactivity requires a bit more discussion.

If an antiserum has been shown to be monospecific and yet reacts with more than one antigen, then it is cross-reactive. Conversely, if an antibody is cross-reactive and if we could obtain a monoclonal serum containing it, the serum should be monospecific and yet reactive with more than one antigen. The way that sera are shown to be monospecific is by running absorption and elution experiments and noticing that all cells which remove any activity from the serum remove all activity. We thus need to model the processes involved in absorption and eluction. In order to do this, we first need some more mathematical notation.

DEFINITION 3.9

(Here we use the conventional immunological notation for absorbed and eluted sera.) If A, B, C, D, R, U, g, and h are given, with $c \in C$ and $d \in D$, then the *absorbed serum* d/c is the serum whose antibody content $h(d/c)$ is $h(d) - g(c)U$, and the *eluted serum* $\mathrm{el}d/c$ is the serum whose antibody content $h(\mathrm{el}d/c)$ is $h(d) \cap g(c)U$.

Example 3.10

If $g(c) = \{a_1, a_2\}$, $h(d) = \{b_1, b_3\}$, and $a_i U b_j$ iff $i = j$, then

$$h(d/c) = h(d) - g(c)U = \{b_1, b_3\} - \{a_1, a_2\}U$$
$$= \{b_1, b_3\} - \{b_1, b_2\} = \{b_3\},$$

and

$$h(\mathrm{el}d/c) = h(d) \cap g(c)U = \{b_1, b_3\} \cap \{a_1, a_2\}U$$
$$= \{b_1, b_3\} \cap \{b_1, b_2\} = \{b_1\}.$$

Note that according to our definition, it will always be the case that $h(d/c) \cup h(\mathrm{el}d/c) = h(d)$. In actual practice this is not always so. When absorption and elution experiments are run, the original serum may sometimes display reactivity found in neither the absorbed nor the eluted sera, and it may sometimes fail to display some of the reactivity found in these sera. This is due to damage to the antibody molecules incurred during absorption and elution and to the increased concentration of an antibody in the absorbed and elated sera, which can cause previously unobserved reactivity to be displayed.

Producing an absorbed or eluted serum is in effect adding a new column to a reaction matrix. However (except in the case of the unexpected reactivities mentioned in the above paragraph), this column has the special property that a serum d will possess exactly those specificities possessed by one or more of its absorbed sera or eluted sera. Thus when examining R-specificities in order to try to determine serological specificities, we want to look only at those R-specificities S such that $d \in \mathrm{Ran}\, S$ iff $d/c \in \mathrm{Ran}\, S$ or $\mathrm{el}\,d/c \in \mathrm{Ran}\, S$. We need to be sure that we can find an R-cover consisting only of such specificities. We prove that this is possible in Theorem 3.13; this theorem is illustrated in Example 3.14. We first need an intermediate result:

LEMMA 3.11

Let $P \subset X \times Y$ be a relation, let T be a P-specificity, and suppose $\mathrm{Dom}\, T \subset Pz$ *for some $z \in Y$. Then S is a P-specificity, where* $\mathrm{Dom}\, S = \mathrm{Dom}\, T$ *and* $\mathrm{Ran}\, S = \mathrm{Ran}\, T \cup \{z\}$. *(We say that T is an* extension *of S to z.) The domain of T may be extended in a similar manner.*

Proof. Suppose xSy. Then $x \in \mathrm{Dom}\, T$, and either $y \in \mathrm{Ran}\, T$ or else $y = z$. If $y \in \mathrm{Ran}\, T$, then xTy, so xPy. If $y = z$, then $x \in \mathrm{Dom}\, T \subset Py$, so xPy. Thus $S \subset P$. The proof for extending the range of T is similar. ■

DEFINITION 3.12

An R-specificity S is *consistent* iff

(1) if $d \in \mathrm{Ran}\, S$, then $d/c \in \mathrm{Ran}\, S$ or $\mathrm{el}\,d/c \in \mathrm{Ran}\, S$ for every cell c such that both d/c and $\mathrm{el}\,d/c$ are in D, and

(2) if $d \in D$ and $d/c \in \mathrm{Ran}\, S$ or $\mathrm{el}\,d/c \in \mathrm{Ran}\, S$, then $d \in \mathrm{Ran}\, S$.

Thus a consistent R-specificity is one which appears in a serum if and only if it appears in the absorbed serum, the corresponding eluate, or both.

THEOREM 3.13

Any R-cover can be used to generate a consistent R-cover of the same cardinality.

Proof. Let $\mathbf{S} = \{S_1, \ldots, S_k\}$ be an R-cover, and let $S_i \in \mathbf{S}$. For each $d \in D$, we perform the following operation on S_i. If $d/c \in \mathrm{Ran}\, S_i$, then $\mathrm{Dom}\, S_1 \subset Rd/c \subset Rd$, and similarly for $\mathrm{el}\,d/c$. In these two cases, we use Lemma 3.11 to extend S_i to d. Otherwise, if $d \in \mathrm{Ran}\, S_i$, $d/c \in D\text{-}\mathrm{Ran}\, S_i$; and $\mathrm{el}\,d/c \in D\text{-}\mathrm{Ran}\, S_i$, then we remove d from $\mathrm{Ran}\, S_i$. If we perform this operation on the $d \in D$ in an order such that we do not perform it on d until after performing it on every $d/c \in D$ and every $\mathrm{el}\,d/c \in D$, then the resulting R-specificity, which we call T_i, is consistent. We let $\mathbf{T} = \{T_1, \ldots, T_k\}$.

We must now show that every $(c,d) \in R$ is in some $T_j \in \mathbf{T}$. We do this by induction on the number of times d has been absorbed and eluted. Let

$(c,d) \in R$. Since **S** is an R-cover, $(c,d) \in S_i$ for some S_i. If there are no elements d/c' or $\mathrm{el}d/c'$ in D, then (c,d) could not have been removed from S_i by the process we went through to create T_i, so $(c,d) \in T_i$. For the case in which there are elements d/c' or $\mathrm{el}d/c'$ in D, we assume as our induction hypothesis that every $(c,d/c) \in T_j$ for some T_j and every $(c,\mathrm{el}d/\mathrm{ic}') \in T_j$ for some T_j.

Case 1. For every c', either $d/c' \notin D$ or $\mathrm{el}d/c' \notin D$. Then (c,d) could not have been removed from S_i by the process we went throught to create T_i, so $(c,d) \in T_i$.

Case 2. For some c', $d/c' \in D$ and $\mathrm{el}d/c' \in D$. Then either $(c,d/c') \in R$ or $(c,\mathrm{el}d/c') \in R$.

Case 2a. $(c,d/c') \in R$. Then by our induction assumption, $(c,d/c') \in T_j$ for some T_j. But because of the process we went through to create T_j, this means that $(c,d) \in T_j$.

Case 2b. $(c,\mathrm{el}d/c') \in R$. As above.

Thus it follows that **T** is an R-cover. ■

Example 3.14. Consider the incidence matrix in Table 1 for a reaction relation R between cells c_1, c_2, c_3, c_4, and c_5 and sera d_1, d_1/c, $\mathrm{el}d_1/c$, d_2, d_3, and d_4. The members S_1, S_2, S_3, S_4 of the R-cover **S** have their elements enclosed by circles, boxes, triangles, and ovals, respectively. $d_1/c \in \mathrm{Ran}\, S_1$, and $\mathrm{el}d_1/c \in \mathrm{Ran}\, S_2$, so S_1 and S_2 can be extended to d_1 by the process of Theorem 3.13. This process also removes d_1 from $\mathrm{Ran}\, S_3$, since neither d_1/c nor $\mathrm{el}d_1/c$ is in $\mathrm{Ran}\, S_3$. S_4 remains unchanged by the process. Thus the resulting R-specificities T_1, T_2, T_3, and T_4, which are represented in Table 2, comprise a consistent R-cover **T**.

Since every induced R-specificity is due to the antigenic content of the cells and sera containing that specificity, we would want every induced R-specificity to be consistent. This is indeed true in our model, as shown by Theorem 3.15 below. This provides additional confirmation of the accuracy of the model.

Table 1
Math Analysis of HLA Serology

	d_1	d_1/c	el d_1/c	d_2	d_3	d_4
c_1	1	1	0	1	0	1
c_2	1	1	0	1	0	1
c_3	1	0	1	1	0	0
c_4	1	0	1	1	1	0
c_5	1	0	1	0	1	1

Table 2

	d_1	d_1/c	el d_1/c	d_2	d_3	d_4
c_1	①	①	0	①	0	△1
c_2	①	①	0	①	0	△1
c_3	1	0	1	1	0	0
c_4	1	0	1	1	1	0
c_5	1	0	1	0	1	△1

THEOREM 3.15

If A, B, C, D, R, U, g, and h are given and S is a U-specificity, then the R-specificity T induced by S is consistent.

Proof. Suppose $d \in \operatorname{Ran} T$. Then there exists $b \in h(d) \cap \operatorname{Ran} S$. If $d/c \in D$ and $\mathrm{el}\, d/c \in D$, then either $b \in d/c$ or $b \in \mathrm{el}\, d/c$, so from the definition of T, either $d/c \in \operatorname{Ran} T$ or $\mathrm{el}\, d/c \in \operatorname{Ran} T$.

Suppose $d/c \in \operatorname{Ran} T$. Then there exists $b \in h(d/c) \cap \operatorname{Ran} S$. If $d \in D$, then $b \in h(d/c) \subset h(d)$, so $d \in \operatorname{Ran} T$ by the definition of T. Similarly for $\mathrm{el}\, d/c$. ■

We conclude this section with an informal discussion of what is meant by *NP-hardness* and *NP-completeness*, and how these concepts relate to the specificity problem.

A problem Q is NP-complete if (1) it is a member of a certain fairly wide class of problems called NP (among other things, this requires that the problem be one requiring a yes-or-no answer), and (2) every other member of NP can be "quickly" reduced to Q. It follows immediately that any NP-complete problem can be quickly reduced to any other NP-complete problem. This means that if a "fast" algorithm were known for the solution of any one of these problems, this would immediately give us fast algorithms for solving every one of them.

We do not wish to be precise about what we mean when we say that an algorithm is "fast" or runs "quickly". Suffice it to say that "slow" algorithms (these are the only ones known for NP-hard and NP-complete problems) are characterized by having large (indeed, exponential) increases in running time for small increases in the amount of data to be handled, whereas "fast" algorithms are characterized by having small (i.e., polynomial) increases in running time under the same conditions.

To date, many NP-complete problems have been discovered, but no algorithm is known for any one of these problems which runs quickly in every instance of the problem (although there are some fast algorithms

which provide approximate solutions to some of these problems). For this and other reasons, it is widely believed that no fast algorithm exists for any NP-complete problem. For a good technical discussion of NP-completeness, we refer the reader to [1], and for a more informal discussion, to [10].

Let Q be the problem of answering the question "given a reaction matrix R and a positive integer k, does there exist an R-cover (or equivalently, a consistent R-cover) of size k?". In [12] we prove that Q is NP-complete by finding a "quick" reduction of the set basis problem [15] to Q. In order to avoid a detailed discussion of a number of concepts from the field of computational complexity, we do not include the proof here.

The problem of actually finding an R-cover of size k, as well as the problem of finding the smallest R-cover, is at least as hard as Q, for given an answer to either of these problems, we could immediately answer Q. Thus everything we have said about the difficulty of NP-complete problems applies equally well to these two problems. However, these two problems may be even harder than NP-complete problems [9]. Thus the term used to describe them is "NP-hard".

The import of the above discussion is that there is strong evidence that the problem of isolating specificities (and thus antigens and antibodies) on the basis of a reaction matrix does not admit any practical algorithmic solution. This provides justification for approaching the problem not in an algorithmic manner, but rather in a heuristic, intuitive one (e.g. by using human-computer interaction). References [12] and [13] include descriptions of an interactive computer program intended for use in this manner.

In presenting a mathematical model of HLA serology, our objective is to find properties of this model which may be of use in analyzing histocompatibility reaction matrices. In such an analysis it is often helpful to have an idea how many antigens, antibodies, and serological specificities may be required to explain the matrix. Thus we shall present a number of theorems providing bounds on these numbers. The reader should note that as a result of Theorem 3.13, all of the theorems we stated in this section hold just as well if "R-cover" is replaced by "consistent R-cover".

IV. SPECIFICITY CARDINALITY

Most of the theorems in this section present bounds on cardinalities of P-covers for an arbitrary relation P. Since these theorems will usually be used to find bounds on R-covers for cell-serum reaction matrices R, it is first necessary to show that applying the theorems to R will enable the user to find bounds on the number of antigens, antibodies, and serological specificities, which appear not in R, but in the matrix U. Lemma 4.1 and Theorem 4.2 are intended to do this.

We make the observation that if $P \subset X \times Y$ is an arbitrary relation, then we can produce a P-cover by covering each row of P with a single

P-specificity, and we can produce another P-cover by covering each column of P with a single P-specificity. Thus we have the following result.

LEMMA 4.1

If $P \subset X \times Y$ is a relation, then there is a P-cover of cardinality $\min(|X|,|Y|)$.

THEOREM 4.2

If R is a reaction relation and k is the smallest cardinality of any R-cover, then

(i) *there are A, B, U, g, and h explaining R, and there is a U-cover* **S** *such that $|A| = k$, $|B| = k$, and $|\mathbf{S}| = k$, and*

(ii) *for no A, B, U, g, and h explaining R can we have $|A| < k$, $|B| < k$, or $|\mathbf{S}| < k$ for any U-cover* **S**.

Proof. If we let A, B, U, g, h, and **S** be as in Theorem 3.8, (i) is satisfied. If we had A, B, U, g, and h explaining R with $|\mathbf{S}| < k$ for some U-cover **S**, then by Corollary 3.5 we would have an R-cover of cardinality less than k. Furthermore, if $|A| < k$ or $|B| < k$, then by Lemma 4.1 a U-cover **S** with $|\mathbf{S}| < k$ would have to exist. Thus (ii) holds. ∎

Given any reaction relation $R \subset C \times D$, we can use Lemma 4.1 and Theorem 4.2 to provide upper bounds on the numbers of antigens, antibodies, and U-specificities necessary in order that an explanation of R can exist. In particular, we can explain R with as few as $\min(|C|,|D|)$ of each. We now present two other theorems (4.3 and 4.4) which can be used in the same way, to provide lower bounds on these same three quantities.

THEOREM 4.3

Let $P \subset X \times Y$ be a relation, with $X = \{x_1, \ldots, x_m\}$ and $Y = \{y_1, \ldots, y_n\}$. Let p be the number of nonidentical rows of P, and let q be the number of nonidentical columns of P. Then no P-cover has cardinality less than $\log_2 \max(p,q)$.

Proof. Let **S** be a P-cover. If two rows x_iP and x_jP of P are different, it must be the case that $\{S \in \mathbf{S} | x_i \in \operatorname{Dom} S\} \neq \{S \in \mathbf{S} | x_j \in \operatorname{Dom} S\}$. Given $|\mathbf{S}| = k$, there are at most 2^k distinct rows. Thus $\log_2 p \leqslant |\mathbf{S}|$. A similar argument holds for the columns. ∎

THEOREM 4.4

Let $P \subset X \times Y$ be a relation, and define a relation Q over the entry positions in the Boolean incidence matrix for P by $(i,j)Q(m,n)$ iff both x_iPy_j and x_mPy_n but not both x_iPy_n and x_mPy_j. Let N be any set of pairs (i,j) such that uQv whenever u and v are distinct elements of N. Then there is no P-cover of cardinality less than $|N|$.

Proof. Let $(i,j),(m,n) \in N$. Then $(i,j)Q(m,n)$, so there is no P-specificity containing both (x_i,y_j) and (x_m,y_n). By repeating this argument for each pair of elements of N, we find that every P-cover must contain at least one distinct P-specificity for each member of N. ■

Geometrically, Q is a relation over the entries in the Boolean incidence matrix for P which is constructed as follows: Pick any two matrix entries. If either is a zero, then they are not Q-related. If they are both ones, then consider them to be opposite corners of a square. They are Q-related if and only if the two remaining corners are not both ones. (In particular, two entries appearing in the same row or column cannot be Q-related.) Thus finding an N consists of finding a set of one-entries in the matrix such that for each pair of them at least one of the corners in the square determined by them is missing.

We remark that Proposition 3 of Ciftan [6], which bounds the minimum number of antigens and antibodies by the size of any lower triangular submatrix obtained by permuting the rows and columns of P, is a special case of Theorem 4.4, in that it is equivalent to producing a restricted type of N (as we show in [12]). In addition, we can generally get a better bound if we do not restrict N in such a manner, as illustrated in Example 5.8.

We now present a theorem which allows us to improve the bounds given by Lemma 4.1 and Theorem 4.3.

THEOREM 4.5

Let $P \subset X \times Y$ be a relation, and suppose x_0P is the union of some of the other rows of P. Let P' be the restriction of P to $X - \{x_0\}$. Given a P-cover of cardinality k, we can produce a P'-cover of cardinality less than or equal to k, and given a P'-cover of cardinality k, we can produce a P-cover of cardinality k. A similar result holds for any column P_y which is the union of other columns of P.

Proof. Suppose $\mathbf{S} = \{S_1, \ldots, S_k\}$ is a P-cover. For $i = 1, \ldots, k$, let $T_i = (\operatorname{Dom} S_i - \{x_0\}) \times \operatorname{Ran} S_i$. Then $\mathbf{T} = \{T_1, \ldots, T_k\}$ is a P'-cover of cardinality no greater than k (although it may be less than k, since some of the T_i may be equal or some $\operatorname{Dom} S_i = \{x_0\}$).

Suppose $\mathbf{T} = \{T_1, \ldots, T_k\}$ is a P'-cover. Let $X' = \{x \in X \mid xP \subset x_0P\}$. By the hypothesis of our theorem, $\cup_{x \in X'} xP = x_0P$. Since $\mathbf{T}$ is a P'-cover, it follows that for every $x \in X$, $\cup \{\operatorname{Ran} T \mid x \in \operatorname{Dom} T\} = xP$. Let $\mathbf{T}' = \{T \in \mathbf{T} \mid x \in \operatorname{Dom} T$ for some $x \in X'\}$. Then $\operatorname{Ran} T \subset x_0P$ for every $T \in \mathbf{T}'$. Therefore by Lemma 3.11 we can extend each T in $\mathbf{T}'$ to a T' containing x. But since $\cup_{T \in \mathbf{T}'} \operatorname{Ran} T = \cup_{x \in X'} xP = x_0P$, x_0P is covered completely by the T', and thus if we let $\mathbf{S} = \{S_1, \ldots, S_k\}$, where $S_i = T_i'$ if $T_i \in \mathbf{T}'$ and $S_i = T_i$ otherwise, then $\mathbf{S}$ is a P-cover. ■

We can use this theorem to repeatedly add or delete unions of rows and columns from a reaction matrix while preserving essentially the same specificity cover. Thus for a given matrix we can improve the bounds given by Lemma 4.1 by removing as many such rows and columns as possible, and we can improve the bounds given by Theorem 4.2 by adding as many such nonidentical rows and columns as possible.

We now discuss a technique which can sometimes be used to break up a reaction matrix into smaller parts which can be analyzed independently.

DEFINITION 4.6

Let $P \subset X \times Y$ be a relation, and let $Q \subset P$. Q is *P-isolated* if $xP = xQ$ whenever xQ $\varnothing$ and $Py = Qy$ whenever $Qy \neq \varnothing$.

Geometrically, Q in the above definition is (not necessarily contiguous) submatrix of P, such that each row or column intersecting the submatrix has its "one" elements only inside the submatrix.

THEOREM 4.7

If P is a relation, $Q_1 \subset P$ is P-isolated, and $Q_2 = P\text{-}Q_1$, then

(i) *Q_2 is P-isolated, and*

(ii) *every P-specificity is either a Q_1-specificity or a Q_2-specificity but not both (except in the case of the null specificity).*

Proof. If $xQ_1 \neq \varnothing$, then $xQ_2 = xP - xQ_1 = \varnothing$. Therefore if $xQ_2 \neq \varnothing$, we must have $xQ_1 = \varnothing$. But $xP = xQ_1 \cup xQ_2$, so if $xQ_2 \neq \varnothing$ we have $xP = xQ_2$. Similarly, if $Q_2 y \neq \varnothing$, $Py = Q_2 y$. Therefore (i) holds.

If $S \neq 0$ is a Q_2-specificity, then $S \subset Q_2 = P - Q_1$, so $S \not\subset Q_1$. Thus a non-null specificity cannot be both a Q_1-specificity and a Q_2-specificity.

If $S \neq \varnothing$ is a P-specificity which is neither a Q_1- nor a Q_2-specificity, then $S \not\subset Q_2$, so $S - Q_2 \neq \varnothing$, so since $S \subset P$, we have $S \cap (P - Q_2) \neq \varnothing$, i.e., $S \cap Q_1 \neq \varnothing$. Similarly, $S \cap Q_2 \neq \varnothing$. Thus there is some $(x_1, y_1) \in Q_1 \cap S$, and some $(x_2, y_2) \in Q_2 \cap S$. Since S is a Cartesian product, $(x_1, y_2) \in S \subset P$. Since Q_1 is P-isolated, $x_1 Q_1 \neq \varnothing$ implies that $(x_2, y_2) \in Q_1$. Similarly, $Q_2 y_2 \neq \varnothing$ implies that $(x_2, y_2) \in Q_2$, which is impossible, since $Q_2 = P - Q_1$. ■

The import of Theorem 4.7 is that if we can divide a relation P into two P-isolated subrelations Q_1 and Q_2, then the specificities we get by analyzing P are the same ones we will get if we analyze Q_1 and Q_2 separately.

In most cell-serum reaction matrices R, it will be unlikely that R has any R-isolated subrelations other than R itself. However, if we can come up with a fast (i.e., polynomial-time) algorithm to discover the R-isolated subrelations if they exist, it will be well worthwhile to use it, since with any NP-hard problem such as the specificity covering problem, even small

reductions in the problem size can lead to large reductions in the amount of work required to solve the problem. We now develop such an algorithm.

THEOREM 4.8

Let $P \subset X \times Y$ be a relation, and define $\{X_i\}_{i=1}^{\infty}$ and $\{Y_i\}_{i=1}^{\infty}$ recursively as follows:

$$X_1 = \{x\} \quad \text{for some} \quad x \in X,$$
$$Y_i = X_i P \quad \text{for} \quad i \geqslant 1,$$

and

$$X_i = PY_i \quad \text{for} \quad i \geqslant 2.$$

Then $Q = [(\cup_i X_i) \times (\cup_i Y_i)] \cap P$ is P-isolated.

Proof. Suppose $xQ \neq \varnothing$. Then $x \in \cup_i X_i$ and $x \in X_{i_0}$ for some i_0. Thus $xP \subset Y_{i_0+1} \subset \cup_i Y_i$ and $xQ = xP$. Similarly, if $Qy \neq \varnothing$, $Qy = Py$. ■

We now present an algorithm based on Theorem 4.8 which divides P up into subrelations of a relation P which may be analyzed independently.

Step 1. Use Theorem 4.5 to remove rows and columns of P which are unions of other rows and columns. Use the theorem repeatedly until no more improvement is obtainable, and call the result P'. The value of this is that if we have removed any rows or columns which are unions of two or more others, P' will have more isolated subrelations than P.

Step 2. Take any row of P', and let M be the set of all "one" elements in the row. Alternately repeat steps 2a and 2b until no additional lines are drawn. The set of "one" elements covered by all of the lines is a minimal P'-isolated subrelation; i.e., it contains no smaller P'-isolated subrelations.

Step 2a. Draw lines through all columns of P' through which lines have not already been drawn and which also contain elements in M. Replace M by the set of all new "one" elements which are thereby covered.

Step 2b. Draw lines through all rows of P' through which lines have not already been drawn and which also contain elements in M. Replace M by the set of all new "one" elements which are thereby covered.

Step 3. Let Q be what is left of P' after the removal of the subrelation found in step 2. By Theorem 4.8, Q is P'-isolated. Q may contain Q'-isolated subrelations, and trivially these are also P'-isolated. Thus we can extract additional P'-isolated subrelations by replacing P' with Q and going through step 2 again.

Note that the above algorithm produces all of the minimal P'-isolated subrelations with at most $2m$ executions of step 2a and $2n$ executions of

step 2b, where m and n are the numbers of rows and columns, respectively, in P'.

V. AN ORDER-THEORETIC APPROACH

The results in this section provide a geometric sort of approach to the problem of finding a minimal specificity cover for a reaction matrix, and they complement the results of the previous sections. We shall state them in a manner requiring the least amount of definitions and background. For generalization and extensions, see [11].

Let A be a set of antigens, and consider the set containing every antibody which attacks at least one antigen of A. According to our mathematical notation, this set is AR. Note that not every set of antibodies can be expressed in this way; i.e., there may be some sets of antibodies which do not happen to be the set of *all* antibodies attacking *any* particular sets of antigens. If a set of antibodies *can* be expressed as AR for some A, we say that it is a member of the *row space* of R. We formalize this below.

DEFINITION 5.1

Let $P \subset X \times Y$ be a relation. The *row space* of P, $\mathbf{R}(P)$, is defined to be $\{AP | A \subset X\}$. Note that $\varnothing \in \mathbf{R}(P)$.

Remark. The column space of P can be defined similarly. However, the results of this section remain the same regardless whether we use the row or column space, so for simplicitly we will not mention the column space. For more details see [11].

DEFINITION 5.2

Let V and W be two collections of sets. An *embedding* of V into W is a map $f: V \to W$ such that for $X, Y \in V$, $X \subset Y$ iff $f(X) \subset f(Y)$. Note that embeddings are 1-to-1, i.e., $f(X) = f(Y)$ iff $X = Y$.

We shall now present a theorem giving a necessary and sufficient condition for an antigen-antibody reaction matrix to explain a given cell-serum reaction matrix. This theorem appears as Theorem 6.2 in [11]. Note that the theorem allows one to construct explicitly the various mapping mentioned.

The import of the theorem is that a given cell-serum reaction matrix $R \subset C \times D$ can be explained in terms of an antibody-antigen reaction matrix $U \subset A \times B$ (as in Sec. III) if and only if every set of sera in the row space of R can be put in correspondence with a set of antibodies in the row space of U in such a way that the correspondence is an embedding. Note that in general, this embedding is not necessarily the "natural" mapping which corresponds each set of sera to the antibodies contained by the sera.

THEOREM 5.3

Let A, B, C, D be sets and $U \subset A \times B$ and $R \subset C \times D$ relations. The following are equivalent:

(i) *There exist maps $g: C \to 2^A$, $h: D \to 2^B$ such that for all $c \in C$ and $d \in D$, cRd iff $[g(c)U] \cap h(d) \neq \varnothing$. (Thus U can be used to explain R as in Sec. III.)*

(ii) *There exists an embedding $f: \mathbf{R}(R) \to \mathbf{R}(U)$.*

Proof. (i) *implies* (ii): For $C_1 \subset C$, let $f(C_1R) = g(\overline{C}_1)U$, where $\overline{C}_1 = \{c \in A | cR \subset C_1R\}$. Note that f is well defined, since $C_1R = C_2R$ implies $\overline{C}_1 = \overline{C}_2$. If $C_1R \subset C_2R$, $\overline{C}_1 \subset \overline{C}_2$, whence $f(C_1R) \subset f(C_2R)$.

Conversely, suppose $f(C_1R) \subset f(C_2R)$. If $d \in C_1R$, then for some $c \in C_1$, cRd and thus $[g(c)U] \cap h(d) \neq \varnothing$. Thus $f(C_1R) \cap h(d) \neq \varnothing$, and thus $f(C_2R) \cap h(d) \neq \varnothing$. Hence, for some $c' \in \overline{C}_2$, $g(c')U \cap h(d) \neq \varnothing$, so $c'Rd$, whence $d \in c'R \subset C_2R$. Thus $C_1R \subset C_2R$.

(ii) *implies* (i): For $c \in C$, let $g(c) = \{a \in A | aU \subset f(cR)\}$. Thus $g(c)U = f(cR)$. For $d \in D$, let $h(d) = B - \cup_{c' \notin Rd} f(c'R)$. Clearly if $(g(c)U) \cap h(d) = f(cR) \cap [B - \cup_{c' \in Rd} f(c'R)] \neq \varnothing$, then cRd.

Suppose cRd, but $f(cR) \cap h(d) = \varnothing$. Then $f(cR) \subset \cup_{c' \notin Rd} f(c'R) \subset f(TR)$, where $T = C - Rd$, since $c'R \subset TR$ and $f(c'R) \subset f(TR)$ for all $c' \in T$. Since f is an embedding, $cR \subset TR$, which is impossible, since cRd, but $d \notin TR$. Thus $[g(c)U] \cap h(d) \neq \varnothing$. ■

In the following material, we shall often be concerned with embeddings into sets of a certain size. The following notation provides a convenient way to generate finite sets of arbitrary size.

NOTATION 5.4

We use $\underline{k}$ to denote the set $\{1, \ldots, k\}$.

In the following theorem, we relate the material on specificity covers to this the material in this section. To try to motivate the theorem for the nonmathematical reader, we preface it with the following paragraph.

Suppose R is a reaction matrix with a specificity cover $\{S_1, \ldots, S_k\}$. Each element of the row space of R is a union of some of the rows of R, and thus may be expressed as the union of the ranges of some of the specificities of R. If we take the set of indices of these specificities and create a map which maps our row space element to this set, we have an embedding from $\mathbf{R}(R)$ to $2^{\underline{k}}$. Conversely, if we can embed $\mathbf{R}(R)$ in $2^{\underline{k}}$, we can reverse this process to obtain a specificity cover $\{S_1, \ldots, S_k\}$ for R.

THEOREM 5.5

Let C, D be sets and $R \subset C \times D$ a relation (see Sec. III). The following are equivalent:

(i) *There exists an R-cover,* $\mathbf{S}=\{S_1,\ldots,S_k\}$, *of cardinality k.*
(ii) *There exists an embedding* $f:\mathbf{R}(R)\to 2^{\underline{k}}$.

Proof. (i) *implies* (ii): Let $f:\mathbf{R}(R)\to 2^{\underline{k}}$ be given by $f(C_1R)=\{S_i|\operatorname{Ran} S_i \subset cR \subset C_1R$ for some $c \in C\}$. If $C_1R \subset R$, then obviously $f(C_1R)\subset f(C_2R)$.
Observe that $R=\cup_{i=1}^{k} S_i$, whence $C_1R=\cup_{i\in f(C_1R)}\operatorname{Ran} S_i$. Thus if $f(C_1R)\subset f(C_2R)$, then $C_1R\subset C_2R$ and f is an embedding.
(ii) *implies* (i): For each $i\in \underline{k}$, let $C_i=\{c\in C|i\in f(cR)\}$, $D_i=\cap_{c\in C_i} cR$, and $S_i=C_i\times D_i$. If $(c,d)\in S_i$, then clearly cRd. Thus $\cup_{i=1}^{k} S_i\subset R$. If cRd, it remains to show that $(c,d)\in S_i$ for some i in $\underline{k}$.
Suppose that cRd but $(c,d)\not\in S_i$ for all i, in particular $d\not\in D_i$ for all i such that $c\in C_i$. For each $i\in f(cR)$, there exists $c_i\in C_i$ such that $d\not\in c_iR$. Let $X=\{c_i|i\in f(cR)\}$. Note that $d\not\in XR$.
Furthermore, $XR\supset c_iR$ for all $i\in f(cR)$, and thus $f(XR)\supset \cup_{i\in f(cR)} f(c_iR)\supset f(cR)$, since $i\in c_iR$. Since f is an embedding, $XR\supset cR$, which is impossible, since $d\not\in XR$ but $d\in cR$. Thus $\cup_{i=1}^{k} S_i=R$. ■

We now show how this material can be used to derive results which we derived earlier by different methods. First we derive the central poritions of the first part of Theorem 4.2.

COROLLARY 5.6

Let R be a reaction relation. Then R can be explained by the $k\times k$ identity matrix, where k is the smallest possible cardinality of any R-cover.

Proof. By Theorem 5.5, $\mathbf{R}(R)$ can be embedded into $2^{\underline{k}}$, whence by Theorem 5.3, R can be explained by the $k\times k$ identity matrix, I_k, since $\mathbf{R}(I_k)=2^{\underline{k}}$. ■

We now derive the central porition of the second part of Theorem 4.2.

COROLLARY 5.7

Let R be a reaction relation and $U\subset A\times B$ a relation which explains R. Then $|A|,|B|\geqslant k$, where k is the smallest possible cardinality of any R-cover.

Proof. Since U explains R, we have an embedding of $\mathbf{R}(R)$ into $\mathbf{R}(U)$ by Theorem 5.3. From Lemma 4.1 and Theorem 5.5 we know that we have embeddings of $\mathbf{R}(U)$, and hence $\mathbf{R}(R)$, into 2^A and 2^B. By Theorem 5.5, k is the least integer such that $\mathbf{R}(U)$ can be embedded in $2^{\underline{k}}$. Thus $|A|,|B|\geqslant k$. ■

We now present an alternative characterization of Theorem 4.3.

COROLLARY 5.8

Let R be a reaction relation with k (j) distinct nonempty rows (columns). Then any R-cover must contain at least $\log_2 k$ ($\log_2 j$) *specificities.*

Proof. We only prove the result for rows, since the result for columns follows similarly. R has a cover with p elements iff $\mathbf{R}(R)$ can be embedded into $2^{\underline{p}}$. Since distinct nonempty rows of R are mapped into distinct nonempty subsets of $2^{\underline{p}}$, and since $2^{\underline{p}}$ has exactly 2^p distinct nonempty subsets, $k \leqslant 2^p$, whence $p \geqslant \log_2 k$. ■

Example 5.9. We now analyze a reaction matrix R which was incorrectly analyzed by Ciftan [6, p. 493]. We analyze it in various ways and show that the order-theoretic approach solves the problem of finding a minimal specificity cover for this matrix.

Let n be a positive integer $\geqslant 2$ *and* $R = \{(i,j) \mid i,j \in \underline{n},\ i \neq j\} \subset \underline{n} \times \underline{n}$. *Thus R, considered as a matrix, has 0's on the diagonal and 1's every place else. Note that* $\mathbf{R}(R)$ *has* $n+2$ *elements,* $\varnothing$, $\underline{n}-\{1\}$, $\underline{n}-\{2\}, \ldots, \underline{n}-\{n\}$ *and* $\underline{n}$; *it is illustrated in Fig. 1.*

By Theorem 5.4, there exists a k-element R-cover iff $\mathbf{R}(R)$ can be embedded into $2^{\underline{k}}$. To embed $\mathbf{R}(R)$ into $2^{\underline{k}}$, we can map $\underline{n}$ to $\underline{k}$, $\varnothing$ to $\varnothing$, and the set $\{\underline{n}-\{i\} \mid i \in \underline{n}\}$ in a 1-1 manner onto n elements $x_1, \ldots, x_n$ of $2^{\underline{k}}$ such that $X_1 \not\subset X_j$ for $i,j \in \underline{n}$ and $i \neq j$. By Sperner's lemma [3, p. 99], we can find n such elements in $2^{\underline{k}}$ iff

$$n \leqslant \binom{k}{\lfloor k/2 \rfloor}$$

(where $\lfloor k/2 \rfloor$ is the greatest integer not exceeding $k/2$). Thus the minimal

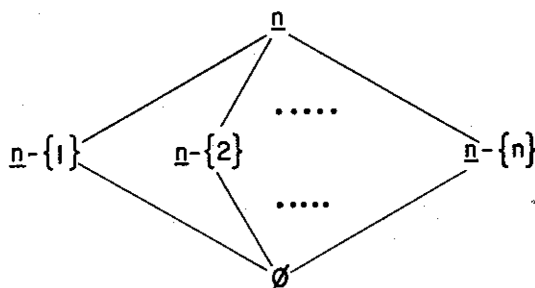

FIG. 1.

specificity cover for R has cardinality

$$\min\left\{k \mid n \leqslant \binom{k}{\lfloor k/2 \rfloor}\right\}.$$

As $n \to \infty$, $k \to \log_2 n$, which is close to the lower bound in Theorem 4.3.

Note that Ciftan [6, p. 493] states that n mutually exclusive antigens are required to explain R. Our analysis shows that many fewer are sufficient.

At this point it might be instructive to compare the lower bounds of Theorems 4.3 and 4.4 and Ciftan's Proposition 3 (see Sec. IV) with the exact quantity calculated above. It is easy to see that Ciftan's result gives a lower bound of 2 on the number of specificities required in the R-cover above. Theorem 4.4 gives a lower bound of 3, while Theorem 4.3 gives a lower bound of $\log_2 n$.

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