were sprayed the very instant they descended from their perch and landed on the floor of the cage beside the insects. Other times they hopped around and inspected the walkingsticks at close range, only to be sprayed just before initiating the actual pecking (Fig. 3). At the instant of discharge, the bird was always closer than an estimated 20 cm from the insect and therefore well within the usual range (30 to 40 cm) of the spray. Whatever sensory input *Anisomorpha* relies upon in "recognizing" and " getting its bearings" on the approaching bird, it is clear that no crude combination of vibrational and visual cues is involved. In the laboratory, attempts to elicit discharges by waving objects in the vicinity of walkingsticks, or by tapping the substrate around them, or by doing both these things simultaneously, almost always met with failure. In the field, I have on rare occasions been sprayed on the hand while reaching to seize a walkingstick, and in crowded laboratory cages individuals sometimes spray when the cages are merely jolted or opened, but as a rule the animals never discharge until they are touched. A jay is evidently "betrayed" from a distance by peculiar characteristics of its own.

When hit by the spray, a jay typically jumps back, shakes its head vigorously, and attempts to cleanse it by wiping it against the plumage on its back; it then flees to its perch. Some secretion inevitably hits the eyes, and for seconds or even minutes thereafter the nictitating membranes are seen to be drawn back and forth over the eyeballs in a rapid wiping action (Fig. 1*D*). All three jays tested were quick to learn to discriminate against *Anisomorpha*. Even when consecutive trials with the same jay were spaced at intervals of 2 to 3 weeks, the bird sometimes remained on its perch and refused to attack.

Anisomorpha is a nocturnal herbivore, yet birds might be among its chief natural enemies. In the environs of Lake Placid, Florida, where I have observed the insects during summer, they are occasionally very abundant and may form dense aggregations feeding on various shrubs at night. They continue feeding until well after dawn, and are then clearly silhouetted against their food plants at a time when bird predation is at a peak. Later in the day they seek shelter from the scorching sun by moving to the base of the plants, only to emerge again after dark.

Anisomorpha is already endowed with secretion and able to spray when it hatches from the egg. In the laboratory, newly hatched nymphs effectively repelled single attacking ants (*Pogonomyrmex badius*). Since the eggs normally hatch at ground level, where foraging ants usually abound, this "inborn" defensive capability must be a major adaptive asset.

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Serologic Codes: Interpretation of Immunogenetic Systems

Abstract. *Transformations of serologic reaction patterns into verbal codes are analyzed. Two equally consistent and complementary models are compared. A model based on the assumption that antibodies are complex (cross-reacting) permits simpler, more uniform, and less prejudicing interpretations of immunogenetic systems than when antibodies are regarded as simple (specific).*

The interpretation of serologic reactions is founded on many assumptions, the arbitrariness of which is not always recognized. It is frequently assumed that there is a one-to-one relation between standard typing reagents and their corresponding antigens. When this assumption proves invalid, complexities of the antigens are usually assumed. However, the same reaction pattern may instead indicate complexities of the antibodies or complexities of both antibodies and antigens (*1*).

The existence of complex antigens (antigens with more than one kind of "antigenic determinants," "factors," or "combining sites") as well as complex antibodies (antibodies able to "cross-react" with antigens which with other antibodies appear to be serologically unrelated) is well documented (*2*, *3*).

The ability of one kind of antibody molecules to react with two serologically different antigens can consequently be symbolically expressed in at least two different ways which I call simple-complex and complex-simple. According to the simple-complex code, the simple (specific) antibody (anti-Q) reacts with the complex (related) antigens QT and QU because these antigens have a property in common. According to the complex-simple code it is instead stated that the complex (cross-reacting) antibody (anti-ab) reacts with the simple (unrelated) antigens a and b because the antibody is cross-reacting (Table 1).

Evidently these two interpretations are equally consistent with the observed reaction patterns (+ + − and + − +) in Table 1. The apparent discrepancy between the interpretations is due only to two different methods of codifying the reaction patterns into verbal symbols.

For the simple-complex code a one-letter symbol is assigned to the *antibodies* (anti-Q, anti-T, anti-U), and the antigens are labeled QT (+ + −) and QU (+ − +) corresponding to their ability to react with the antibodies. In contrast, for the complex-simple code a one-letter symbol is assigned to the *antigens* (a and b), and the antibodies are labeled anti-ab (+ +), anti-a (+ −), and anti-b (− +) corresponding to their ability to react with the two antigens.

Both models give oversimplified and conceptually different pictures of reality. The conventional simple-complex model is idealized (and thus falsified) in one direction in that simple (specific) antibodies are assumed. The new complex-simple model is idealized (and thus falsified) in the opposite direction, in that simple antigens (antigens with only one kind of antigenic determinants) are assumed. Both of these models are consequently restricted and complementary.

In order to classify and discuss quali-

tatively the concept of complex (cross-reacting) antibodies which may show continuous variations in cross-reacting ability with related antigens, it is necessary to introduce hypothetical discontinuous serologic elements (antibody specificities) which cannot be subdivided and do not cross-react. The relation of antibody specificities to other serologic units is evident from the following definitions:

Antigenic determinant. The simplest serologic element which either alone or in combination with elements of the same or different kinds constitutes an antigen and reacts with antibodies.

Simple antigen. A structure with antigenic determinants of only one kind. When only simple antigens are considered, the terms antigenic determinant and antigen are interchangeable.

Complex antigen. A structure with antigenic determinants of more than one kind.

Antibody specificity. A property of an antibody, which causes it to react with one and only one kind of antigenic determinant. No obligatory relation exists between antibody specificities induced by immunization with an antigen and its antigenic determinants. Thus, a simple antigen having only the antigenic determinant a may induce the formation of a simple antibody with the specificity anti-a and "cross-reacting" antibody molecules with the combined antibody specificities anti-abc, anti-ab, anti-ac, but usually no antibody molecules lacking the anti-a specificity such as anti-bc, anti-b, or anti-c.

Simple antibody. An antibody molecule which reacts with one and only one kind of antigenic determinant and accordingly has only one kind of antibody specificity. When only simple antibodies are considered, the terms antibody specificity and antibody are effectively interchangeable.

Complex antibody. An antibody molecule such as anti-ab, which reacts with more than one kind of antigenic determinant (a and b) and accordingly has more than one antibody specificity.

Pure antiserum. An antiserum which has only one kind of antibody molecules. These antibody molecules may be simple (anti-a) or complex (anti-ab).

Mixed antiserum. An antiserum which has two or more physically separable kinds of antibody molecules such as anti-a + anti-b + anti-ab.

To illustrate further the difference between these two complementary models of classification, a comparison is made in Table 2 of the Fisher-Race simple-complex model of the Rh system (*3*) with the new complex-simple model.

Table 1. Comparison between simple-complex (QTU-terminology) and complex-simple (ab-terminology) interpretations.

Reactions with			Interpretation (phenotypes)	
anti-Q anti-ab	anti-T anti-a	anti-U anti-b	Simple-complex	Complex-simple
+	+	−	OT	a
+	−	+	QU	b

Although this crude complex-simple model is given primarily in order to illustrate the basic differences between a simple-complex and a complex-simple interpretation, the complex-simple model also permits better understanding of some of the following complexities thought to exist in the Rh system when the Fisher-Race model is used.

Antiserums have been found, which react with "compound" Rh antigens. According to the aforementioned complex-simple interpretation, these reagents can be denoted anti-ae in the case of anti-CE, anti-bf(anti-Ce), and anti-dh(anti-ce or anti-f), and there is no need to assume any *cis* or *trans* position effects by the complex-simple model to account for these "compound antigens" (*3*). In addition, the complex-simple model in Table 2 permits the existence of a total of 255 Rh antiserums, each with its own pattern of reactions due to all combinations of antibody specificities against eight antigenic determinants (a to h inclusive). According to a conventional simple-complex interpretation, these antiserums may have been thought to reveal up to 255 different Rh antigens.

The complementarity of the restricted simple-complex and complex-simple models is illustrated by the fact that, whereas the complex-simple model permits a simplification in the concept of compound antigens, the simple-complex model permits a better understanding of the selective blocking effect of incomplete anti-D serums. Certain phenomena such as formation of anti-D in Rh(+) individuals cannot be accounted for by either of these oversimplified models, but they are explainable by more sophisticated complex-simple or complex-complex models (*1*, *4*).

A comparison of a complex-simple immunogenetic system with the conclusions reached if the system is incorrectly interpreted as being simple-complex is given in Table 3.

In this idealized immunogenetic system only simple antigens are considered. To simplify the discussion further, a one-to-one relation exists between antigenic determinants and the corresponding genetic units, which, also for the sake of simplicity, are regarded as alleles at the same locus. Thus gene *a* gives rise to antigenic determinant a, gene *b* to antigenic determinant b, and gene *c* to antigenic determinant c. In order to include absence of any detectable antigenic determinants, gene *d*, which can be regarded as a "silent gene," is introduced. Different binary combinations (heterozygotes) of the simple antigens a, b, and c are also included (ab −, a − c, − bc).

No restricting assumptions are made, however, with respect to the antiserums, which (although pure) are permitted to show all conceivable degrees of cross-reactions and overlapping reactions with the three simple antigens a, b, and c. Accordingly, within this complex-simple immunogenetic system a maximum of seven qualitatively different, pure typing reagents could exist owing to all combinations of antibody specificities for three antigenic determinants (a, b, and c). Three of these reagents are simple (anti-a, anti-b, and anti-c) and accordingly strictly mono-

Table 2. Comparison between the Fisher-Race interpretation of the Rh system (simple-complex interpretation) and a complex-simple interpretation for this system (a, b . . . h-terminology).

Reactions with						Antigenic composition	
anti-D anti-abcd	anti-C anti-abef	anti-E anti-aceg	anti-c anti-cdgh	anti-e anti-bdfh	anti-f anti-dh	Simple-complex	Complex-simple
+	+	+	−	−	−	CDE	a
+	+	−	−	+	−	CDe	b
+	−	+	+	−	−	cDE	c
+	−	−	+	+	+	cDe	d
−	+	+	−	−	−	CdE	e
−	+	−	−	+	−	Cde	f
−	−	+	+	−	−	cdE	g
−	−	−	+	+	+	cde	h

Table 3. Comparison of a complex-simple immunogenetic system (left side) with the conclusions reached if the system is incorrectly interpreted as being simple-complex (right side). The reactions of three simple antigens (a, b, and c) with seven pure antiserums (anti-abc, anti-ab, etc.) are considered. The seven antiserums are also labeled anti-P, anti-Q . . . anti-V to illustrate a supposed simple-complex relation. To avoid unnecessary symbols, the terms (ab −) and P Q R − T − − are used to imply the reaction patterns (a + b + c −) and (P + Q + R + S–T + U − V −) according to the correct complex-simple (abcd-terminology) as well as the incorrect simple-complex (P Q R . . . V-terminology) interpretations.

Actual case		Reactions with antiserums							Incorrect interpretation	
Complex-simple		anti-abc	anti-ab	anti-ac	anti-bc	anti-a	anti-b	anti-c	Simple-complex	
Genotype	Phenotype	anti-P	anti-Q	anti-R	anti-S	anti-T	anti-U	anti-V	Phenotype	Genotype
a/a	(a − −)	+	+	+	−	+	−	−	P Q R − T − −	*PQRT/PQRT*
a/d	(a − −)	+	+	+	−	+	−	−	P Q R − T − −	*PQRT/* −
b/b	(− b −)	+	+	−	+	−	+	−	P Q − S − U −	*PQSU/PQSU*
b/d	(− b −)	+	+	−	+	−	+	−	P Q − S − U −	*PQSU/* −
c/c	(− − c)	+	−	+	+	−	−	+	P − R S − − V	*PRSV/PRSV*
c/d	(− − c)	+	−	+	+	−	−	+	P − R S − − V	*PRSV/* −
a/b	(ab −)	+	+	+	+	+	+	−	P Q R S T U −	*PQRT/PQSU*
a/c	(a − c)	+	+	+	+	+	−	+	P Q R S T − V	*PQRT/PRSV*
b/c	(− bc)	+	+	+	+	−	+	+	P Q R S − U V	*PQSU/PRSV*
d/d	(− − −)	−	−	−	−	−	−	−	− − − − − − −	− / −

specific, whereas the remaining four are complex (anti-abc, anti-ab, anti-ac, anti-bc) and accordingly "cross-reacting."

The complex-simple immunogenetic system arbitrarily set up (expressed with abcd-terminology in the left hand side of Table 2) is compared with the conclusions which would be reached if the same system was interpreted as being simple-complex (expressed with P, Q, R, S . . . V-terminology in the right hand side of the Table). The interpretation, based on the restricting assumption that all antibodies are simple, requires the assumption that the antigens are complex and that antigen a is P Q R T, antigen b is P Q S U, and antigen c is P R S V. This assumption leads to a very complicated interpretation of the originally very simple four-allele immunogenetic system. Only a few of these complexities will be briefly mentioned as follows:

1) Instead of three antigens (a, b, and c), there would be at least seven antigens (P, Q, R, S, T, U, and V).

2) Some of these seven antigens would be found to be inherited in groups or complexes (P Q R T, P Q S U, and P R S V) as are the phenogroups in cattle and the Rh antigens or Rh factors in man.

3) Certain pairs of these antigens (Q and R, Q and S, R and S) would occur both as genetically nonsegregating units and segregating elements, as do the C and D, C and E, and D and E antigens in the Rh system (*3*). For example, the Q and R antigens occur together in the nonsegregating phenogroup P Q R T, but as segregating units in phenogroups P Q S U and P R S V.

4) Antigen P is found in all samples having one or more of the Q through V antigens.

5) Antigen U is found only in samples where the Q and S antigens occur as nonsegregating units (phenogroup P Q S U), and this is also found to be the case for the T and V antigens with respect to the QR and RS antigens, respectively. This situation would be similar to the "compound antigens" in the Rh system (*3*) which are explained by *cis*-position effects of, for example, the C and E genes leading to the formation of a "new" antigen (CE).

A simple-complex interpretation of this immunogenetic system would also be affected for example by (i) which antiserums are known and their sequence of discovery, and (ii) the frequencies of the *a, b, c,* and *d* genes in the material or population studied.

This will be briefly illustrated in a situation when only two antiserums are known, each having one antibody specificity in common and, in addition, one unique antibody specificity each. Thus, from Table 3, the antiserums anti-ab (anti-Q) and anti-bc(anti-S) satisfy these requirements.

In Table 4, the simple-complex (QS-terminology) and complex-simple (abcd-terminology) interpretations are compared for these antiserums.

Table 4. Comparison of simple-complex and complex-simple interpretations.

Reactions with		Interpretation		
anti-Q anti-ab	anti-S anti-bc	Simple-complex	Complex-simple	
+	+	(Q + S +)	(a + b + c −)	*a/b*
			(a + b − c +)	*a/c*
			(a − b + c −)	*b/b* or *b/d*
			(a − b + c +)	*b/c*
+	−	(Q + S −)	(a + b − c −)	*a/a* or *a/d*
−	+	(Q − S +)	(a − b − c +)	*c/c* or *c/d*
−	−	(Q − S −)	(a − b − c −)	*d/d*

Thus the supposed relation between the Q and S antigens is determined by the frequencies of the four alleles *a, b, c,* and *d* in the material, sample, or population studied. A few examples will illustrate this point further.

1) In population I, only the gene *b* is common. The two antiserums would be regarded as qualitatively identical and demonstrating a "public" antigen (QS).

2) In population II, only the genes *a* and *c* are common. The two antigens Q and S would now be regarded as "antithetical." According to a simple-complex interpretation it would, however, be difficult to explain why the heterozygote (Q + S +) occurs as a nonsegregating unit in population I. This is thought to be analogous to the occurrence of "nonsegregating" Gm (a + b +) heterozygotes in Negroes, in contrast to the occurrence of segregating γ-globulin heterozygotes in whites (*5*).

3) In population III, only the genes *a, b,* and *c* are common. In this population, there might appear to be an excess of heterozygotes, if the simple-complex (QS) interpretation is applied. Thus all offspring of a parent who is genotypically *b/b* would, according to a simple-complex interpretation, be re-

garded as heterozygotes (Q + S +). This could be analogous to the excess of heterozygotes in the MN system among offspring of some parents who are of blood group MN (*3*).

4) In population IV, only the genes *a*, *b*, and *d* are common. The fact that the S antigen commonly occurs in combination with the Q antigen but is rarely or never expressed in its absence might lead to complicated assumptions of a biochemical or genetic nature regarding the relation between the Q and S antigens, when a simple-complex relation is assumed between pure typing reagents and antigens. This is thought to be analogous to the situation in the Rh-system, where the antigen C rarely occurs in the absence of antigen D.

5) In population V, only the genes *a*, *c*, and *d* are common. This situation is similar to the ABO-system, where (a + c +) = AB, (a + c −) = A, (a − c +) = B, and (a − c −) = O. Rare occurrence of gene *b* could, however, lead to anomalous inheritance; for example, it would be possible for an AB(*b*/*d*) × O(*d*/*d*) mating to have AB(*b*/*d*) or O(*d*/*d*) offspring.

Thus the method of classification drastically affects the interpretation of immunogenetic systems. In this report the influence of a prejudicing method of classification has partially been evaded by introducing a new code of classification. This complex-simple model is based on the introduction of a new fundamental element—the antibody specificity—which cannot be subdivided and does not cross-react. Hence the quantitative phenomenon of cross-reactivity can be discussed in qualitative terms.

Additional consequences of a theory based on the concept that antibodies consist of any combination of a restricted number of discrete antibody specificities are beyond the scope of this report.

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Muscle Volume Changes: Relation to the Active State

Abstract. *The volume of a frog sartorius muscle increases during a single twitch and subsequently decreases. The magnitude of volume change is 10^{-5} cm³ per gram of muscle. The time courses of change in volume and of onset of tension vary with temperature in a similar way, which implies a relation between the volume changes and the contraction mechanism. The character of the change depends upon the initial length of the muscle: the greatest decrease accompanies an isometric twitch at reference length. Treatment of the muscle with an iodide Ringer solution prolongs the active state and the volume change. These results suggest a correlation between the volume changes and the active state.*

Ernst (*1*) has demonstrated a decrease in volume of about 0.002 percent during tetany of isolated striated muscle from the frog. Meyerhof and Hartmann (*2*) showed that the decrease paralleled the development and maintenance of tension in this muscle. The occurrence of an increase in volume preceding the decrease was reported by Fischer (*3*). Most early experiments were performed with frog gastrocnemii in which tetany was induced in order to obtain an observable change in volume. However, Hill (*4*) showed that this decrease may be caused by an internal pressure of 200 mm-Hg developed within the gastrocnemius muscle.

Experiments were performed recently (*5*) with sartorius muscle, in which fibers are oriented parallel to the muscle axis, so that no volume decrease could arise from self-compression forces. The sartorius muscle is quite thin and can readily be supplied with oxygen, whereas contractions of excised gastrocnemius muscle show a noticeable deterioration because of anoxia.

In studies of the volume change in sartorius muscle during an isotonic twitch (*6*) the instrumentation used had a resolution of approximately 10^{-7} ml volume and a time response of about 1 msec. This sensitivity made muscle tetany unnecessary; the change in volume during a twitch was of greater interest than the volume change during tetany, throughout which a complex nonlinear overlap of successive changes occurred. The muscle, contained in a chamber open to the environment, was surrounded by frog Ringer solution (*7*). The solution and a wire close to a region of the solution's surface constituted two plates of a capacitor, the storage capacity of which depended upon the distance between the plates, and therefore upon the total volume of the chamber.

In the experiments reported here, we used a pressure transducer instead of a proximity transducer: the muscle chamber was closed to the environment and the change in pressure of the fluid surrounding the muscle was measured. The volume changes were quite small so that the pressure change in a closed chamber was equivalent to a corresponding volume change in a chamber at atmospheric pressure. This replacement of an isobaric system by an isochoric system resulted in a greater stability of the resting volume (recorded base line).

Measurements taken with a resolution of 10^{-7} ml contained superimposed noise introduced by high-gain amplifier circuitry and by spurious fluctuations at the transducer-fluid interface. A signal-averaging computer was employed to improve the signal-to-noise ratio of the data. Successive wave forms of volume change were digitized and added in a magnetic core memory; as the number of wave forms increased, the random noise in the input tended to average to zero, while the amplitude of the signal rose linearly. In these experiments, voltage wave forms corresponding to volume changes accompanying 16 successive twitches were averaged, with a resultant signal-to-noise ratio improvement of 4 to 1.