mathematics entirely in terms of specific facts and problems. Unfortunately the methods involved in proving these facts involve such a long journey up the ladder of abstraction that it is impossible to give, in any brief article, a fair idea of how they work. Separated from the methods which establish them, facts can convey only a partial picture of mathematics. Like the great temples of some religions, mathematics may be viewed only from the outside by those uninitiated into its mysteries. Anyone who thinks at all about what is involved in asserting that Kervaire's ten-dimensional surface is unsmoothable will sense the power of the methods topologists have developed for organizing our knowledge of space, but understanding these methods is reserved for those who devote years to the study of mathematics.

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The Heterogeneity of the Immune Response

The quantity and nature of antigen can regulate a variety of immunological functions.

Jonathan W. Uhr

The immune response represents a differentiation process in which a small subpopulation of lymphoid cells replicate at an increased rate and synthesize γ-globulin antibodies and perhaps other immune factors. For half a century now several phases of the immune process have been recognized (*1*). First, there is a "latent" period, which is the interval between the first injection of antigen and the commencement of detectable antibody formation. Second, there is the phase of antibody synthesis which lasts for several weeks to many years. Finally, there is the phase of immunological memory, during which there is an enhanced antibody response upon readministration of the specific antigen. This phase of immunological memory usually accompanies antibody synthesis, can occur without it, and usually lasts for years. The enhanced antibody response after reinjection of antigen (the secondary antibody response) has a shorter latent period and a higher peak concentration of serum antibody than the primary response.

During the past decade, there has accumulated considerable new knowledge concerning the cellular and serologic aspects of the immune response. Regarding the cellular aspects, the complexity of the population dynamics of lymphoid cells has become evident. Primary (thymus) and secondary lymphoid organs (lymph nodes, spleen, and so forth) have been described (*2*), and reticuloendothelial cells have been considered necessary for the processing of antigen for the primary antibody response (*3*). It has become apparent that in addition to morphological differences between lymphoid cells, there are striking differences in their response to antigen. Although many lymphocytes encounter antigen in a primary response, only a small fraction of the cells respond by the synthesis of a specific antibody (*4*). There is also a constant exchange of lymphocytes between lymphoid organs and lymph (*5*), and transformation of the small lymphocyte to a large replicating lymphocyte can occur (*5*, *6*).

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Serologists have long recognized that antibody molecules from the same species and of similar specificity can differ in many ways: size (*7*), avidity (*8*), capacity to fix complement (*9*), ability to cross the placenta (*10*), and others, but there has been difficulty in relating biological to physical properties. During the past several years there have been described three different molecular classes of γ-globulin molecules that can be associated with antibody activity, γ_{1A}, γ_{1M}, and γ_2 as defined by immunoelectrophoresis (*11*), and the biological properties of these molecules are now being systematically studied (*12*). In addition, observations have been made concerning the time of appearance of these classes of antibody during immunization.

This increase in our descriptive knowledge of the cellular and serologic events that follow antigenic stimulation has not yet resulted in an understanding of the mechanisms by which the observed events occur, so at the present time the immune response can only be defined in operational terms. In this article the different immune factors produced in response to antigenic stimulation and the influence of type and dose of antigen on various aspects of the immune response will be described, and the possible modes of regulation of the synthesis of these different immune factors will be discussed.

In these studies, bacteriophage ϕX174 (ϕX) has been employed as antigen (*13*) because it presented several advantages over previously used systems. First, the phage is an excellent immunogen and trace amounts (0.1 μg) without the use of adjuvants stimulate the formation of precipitating antibody. Second, the assay for antibody which depends upon the rate of inactivation (k) of phage by a given antiserum is an extremely sensitive and

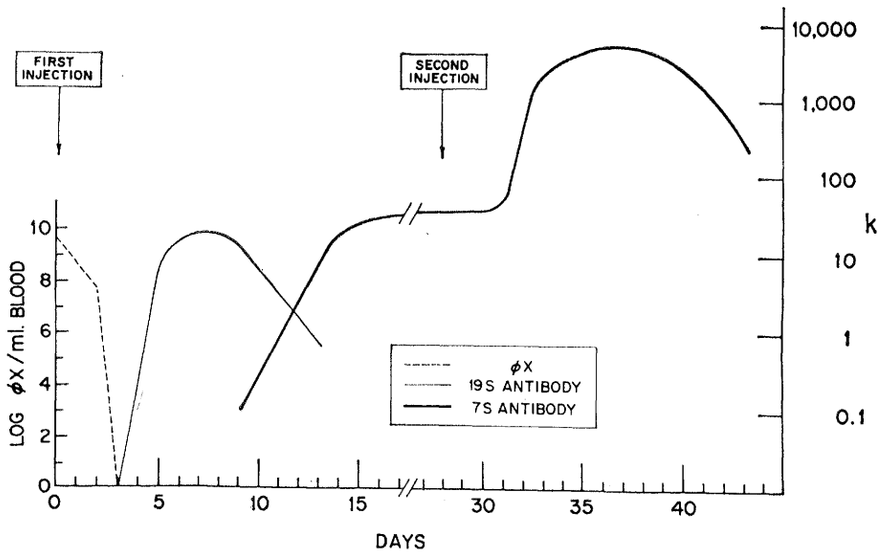

Fig. 1. Antibody response to ϕX in the guinea pig after two intravenous injections of 10^{11} PFU ϕX administered 1 month apart.

quantitative test. Finally, antibody is usually not detected in serums obtained from normal nonimmunized animals. Using this phage as antigen, we have shown that adult mammals (*14*), chickens, frogs, and goldfish (*15*) respond in a generally similar manner to a single injection of 10^{10} PFU (plaque-forming units) of ϕX in saline. (i) Two weeks after injection, the serum k is approximately 10^{3-4} above that before the single injection ($k = 10^{-3}$); (ii) the antibody molecules at this time are predominantly rapidly sedimenting with a sedimentation constant of 19*S*, and they are inactivated by 0.1*M* 2-mercaptoethanol; (iii) several weeks later, these 19*S* antibody molecules are substantially or completely replaced by slowly sedimenting antibody molecules having a sedimentation constant of 7*S*, and these 7*S* molecules in certain mammalian species such as the guinea pig are not inactivated by 2-mercaptoethanol.

A similar sequence in the antibody response was previously reported by Stellos *et al.* (*16*), LoSpalluto *et al.* (*17*), Bauer and Stavitsky (*18*), and Benedict *et al.* (*19*). The inactivation by 2-mercaptoethanol as a means of classification of antibody molecules in the guinea pig has made possible the study of the kinetics of formation of both rapidly (19*S*) and slowly sedimenting (7*S*) antibody. It must be borne in mind, however, that Rocky and Kunkel (*20*) have recently reported an antibody having an intermediate sedimentation constant; this antibody, which was obtained from humans, is inactivated by 2-mercaptoethanol. In nonmammalian vertebrates, 7*S* antibody can also be inactivated by 2-mercaptoethanol (*15*). It is possible, therefore, that exceptions will appear to the 2-mercaptoethanol classification.

Kinetics of Antibody Formation to Bacteriophage ϕX174 in Guinea Pigs

Figure 1 summarizes the phases of antibody formation which have been detected after two intravenous injections into adult guinea pigs of 10^{11} PFU of ϕX administered 1 month apart. These phases are as follows:

"Nonimmune" elimination of antigen. The duration of this phase depends in part upon the dose of antigen injected. Previous studies by others (*21*), in which 1 to 50 mg of protein, trace-labeled with radioactive iodine, were injected into guinea pigs, indicated that the time of onset of immune elimination (accelerated elimination because a sufficient amount of antibody had entered the circulation) varied from 8 to 13 days. With ϕX as antigen, the onset of detectable immune elimination is 55 to 65 hours after injection of 10^{11} PFU of ϕX, 40 to 45 hours after injection of 10^{9} PFU ϕX, and 20 to 30 hours after 10^{7} PFU ϕX (10^{7} PFU of ϕX is approximately 10^{-7} mg). Part of this phase therefore actually represents production of small amounts of antibody that was not detected in reaction with excess antigen. If a latent period exists, it is shorter than 24 hours.

Immune elimination. As has been previously described for labeled heterologous serum proteins (*22*), antigen elimination is accelerated because of the accumulation in the circulation of amounts of antibody sufficient to affect the concentration of phage in the serum by neutralization or by the formation of antigen-antibody complexes that are rapidly phagocytized or both.

Exponential phase of synthesis of 19S antibody. When the log of the concentration of serum antibody is plotted against time, a straight line is obtained; the slope of the line is constant until day 4 to 5 over a wide dose range of phage, provided the dose is sufficient. Since studies by others (*23*) have indicated that serum antibody concentrations accurately reflect antibody synthesis during the phase of rapid increase of serum antibody, we have called this slope the relative rate of antibody formation. It can be conveniently expressed as the length of time needed to double the concentration of antibody in the serum, which, in the foregoing instance, may be as short as 6 to 8 hours. Below a critical dose of antigen, slower relative rates may occur. This phase probably begins within hours after the injection of antigen.

Nonexponential phase of synthesis of 19S antibody. This phase lasts for 4 to 6 days, after which the half-life of 19*S* antibody appears similar to that of passively administered 19*S* antibody in normal animals, namely, approximately 24 hours. This observation indicates that 19*S* synthesis has either stopped or is continuing at rates too low to be detected by this method.

Exponential phase of primary 7S antibody formation. This phase, which is not detected until approximately 1 week after injection of ϕX, lasts for approximately 4 days. The shortest doubling time observed thus far has been 10 hours, but unlike the primary 19*S* response a maximum relative rate has not yet been demonstrated.

Nonexponential 7S antibody synthesis. This phase lasts for at least 2 years, presumably for the life of the animal.

"Latent period" after secondary challenge. A major cause for the delay before detecting an increase in serum antibody results from the difficulty of detecting the formation of new antibody in the presence of high concentrations of serum antibody persisting from the primary response. Another cause is the inhibiting effect of excess

serum antibody on the secondary antibody response.

Exponential phase of synthesis of secondary 7S antibody. This phase lasts until 5 to 6 days after challenge. Small amounts of 19*S* can be produced during this phase but are usually undetected by the 2-mercaptoethanol method. Doubling times of 7 hours have also been obtained during the secondary 7*S* response.

Nonexponential secondary 7S antibody synthesis. This phase lasts for at least 10 days. Unlike the primary 7*S* response, serum antibody usually begins to decrease by 2 weeks at a rapid rate indicating a marked decrease in the absolute rate of antibody formation. Perhaps, during the secondary, but not the primary antibody response, the vast majority of specifically immunized cells have synchronously differentiated into antibody-forming cells and, later, have synchronously ceased to synthesize antibody. Sercarz and Coons (*24*) have previously demonstrated a stage of "exhaustion" after a secondary antibody response.

Generally similar results of antibody-formation kinetics have been obtained recently in rabbits immunized with poliomyelitis virus (*25*) and mice immunized with the RNA bacteriophage F-2 (*26*).

The phases of the immune response to bacteriophage ϕX shown in Fig. 1 are incomplete even in the light of present knowledge. Recent experiments done in collaboration with Dr. B. Benacerraf have indicated that there are both 7*S* γ_1- and γ_2- antibodies to ϕX separable in hyperimmune guinea pig serum by starch-block electrophoresis; thus all three major immunoglobulins participate in the antibody response to ϕX174 in guinea pigs. Since there is no simple quantitative method for separating these two classes of 7*S* γ-globulins, it has not been possible to study the kinetics of their formation.

In addition, 1 to 2 weeks after injection of 10^{10} PFU of ϕX in complete Freund's adjuvant into the footpads of guinea pigs, they can display delayed skin reactivity to this antigen. The reactions appear to be specific to the phage and are not elicited by subtoxic doses of an extract, that had been treated with high frequency sound, of the host bacteria, *Escherichia coli* C. Unfortunately, there is no quantitative assay for this type of immune response.

The foregoing findings, however, have led to certain questions and conclusions concerning the immune response to ϕX. The first is whether the initial injection of phage into adult guinea pigs is a secondary antigenic challenge since the host bacteria *E. coli* C presumably is ubiquitous in nature. This possibility appears improbable because (i) the first response to ϕX is composed predominantly of 19*S* antibody molecules, and the second response of predominantly 7*S* antibody molecules; (ii) adult guinea pig serums are usually without antibody to ϕX; (iii) a similar prompt antibody response to ϕX174 has been obtained in sheep embryos (*27*), newborn humans (*28*), and newborn guinea pigs (*14*). There is no evidence to indicate that either of these three species is routinely immunized during gestation to ϕX or, for that matter, to any antigen. In particular, it is clear that the sheep embryo has not been so immunized because it remains essentially agammaglobulinemic (except for traces of γ_{1M}-globulin) throughout gestation unless experimentally immunized by intrafetal injection of antigen, in which instance, large amounts of γ-globulin as well as specific antibody are synthesized by the fetus (*27*). These findings have led to the conclusion that antibody formation to a first injection of ϕX is usually a primary immunological response.

The second question concerns a "latent" period. By the use of trace amounts of ϕX the potentiality of immune cells to produce 19*S* antibody promptly was not masked by excess antigen. The studies showing shorter lag periods with smaller doses suggest that, if a "latent" period exists, it is no more than several hours in duration and may be due to the necessity of processing antigen before presentation to the lymphoid cell. In contrast, there was a delay of almost 1 week before 7*S* was detected. This delay is probably not due to failure of antigen to meet immunologically competent cells since increasing the dose of phage did not significantly affect this delay.

A third question concerns the factors accounting for the kinetics of the synthesis of antibody to ϕX. There are several possible explanations for doubling times as short as 6 to 8 hours. (i) Antibody-producing cells are dividing at this rate. Defendi and Manson (*29*) have suggested that 11 to 12 hours is the minimum interval between mitoses in mammalian cells. There are, however, recent reports suggesting that plasma cells may divide at a more rapid rate, perhaps every 7 to 8 hours (*30*). (ii) The quality of antibody changes so that the ratio of the neutralizing efficiency of antibody to the weight of antibody protein increases. There can be a striking increase in the binding affinity of antibody during immunization (*8, 31*) but such changes take place over a period of weeks to months and have not yet been observed within the 19*S* system (*32*). It is unlikely that a significant increase in binding affinity would take place over a 7-hour period, but this possibility can only be tested by an assay that does not depend on the quality of antibody, which is the case with quantitative precipitation. (iii) There is continual recruitment of uncommitted cells after contact with antigen. This factor can be evoked for the primary 19*S* response but can be excluded as a significant contribution to the short doubling times observed in the secondary 7*S* response in which a large specifically sensitized cell population already exists. (iv) There is an exponential increase in the absolute rate of antibody formation per cell. Such a situation could arise if there was an exponential increase in a subcellular particle that was rate-limiting with respect to antibody synthesis, for example, replication of ribosomes. No support for this possibility has emerged from studies of γ-globulin synthesis which appears to be due to DNA-dependent RNA synthesis (*33*). (v) There is a linear increase in the absolute rate of antibody formation per cell. If the dominant contribution to the increasing absolute rate of antibody synthesis is cell replication, an apparent exponential increase can result.

Although there is no further evidence at present to allow a definitive choice between the foregoing possibilities, morphologic and histochemical studies clearly indicate that a differentiation process takes place in the development of the plasma cell. During differentiation there is an increase in the amount and organization of the endoplasmic reticulum (*34*) and an increase in intracellular γ-globulin (*35*). These observations support the possibility of either exponential or linear increase and suggest that the absolute rate of antibody formation per cell is increasing during the "exponential" phase of 19*S* and secondary 7*S* formation.

Table 1. Immune functions affected by dose of antigen.

Function	Dose of ϕX affecting function (PFU)
Primary 19*S* formation	$>10^7$
Relative rate of 19*S* formation	10^7–10^9
Maximum relative rate of 19*S* formation without 7*S* formation	10^9
7*S*-immunological memory	$1–3 \times 10^9$ (+endotoxin?)
Primary 7*S* formation	$\geq 3 \times 10^9$
Relative rate of primary 7*S* formation	3×10^9–$>10^{11}$
Relative rate of secondary 7*S* formation	function of $\frac{\text{secondary dose}}{\text{primary dose}}$

Morphologic studies and studies with tritiated thymidine (*36*) also have shown that immature plasma cells replicate while mature plasma cells do not. Thus, to summarize, the following cellular events probably underly the kinetics of formation of antibody to ϕX. After a delay of no more than several hours after antigen injection, cells begin to differentiate (form 19*S* antibody) and also replicate, probably at maximum rates; the relative rate of antibody formation is a function of these two rates; at the end of the exponential phase, replication stops, but differentiation into mature plasma cells continues; at approximately 10 days, synthesis of 19*S* antibody stops. In contrast, there is a lag period of several days before a population of cells forming 7*S* antibody undergoes a similar series of cellular events; this population of cells that forms 7*S* antibody and has 7*S*-immunological memory remains intact for the lifetime of the animal. This explanation does not exclude the possibility that the same cell is responsible for both 19*S* and the later 7*S* antibody synthesis.

Immunological Memory

The short-lived nature of the 19*S* antibody response to ϕX and the absence of substantial amounts of 19*S* antibody in the secondary antibody response suggested that 19*S* antibody formation might not lead to the development of a *persisting* immunological memory. That a small dose of ϕX (10^9 PFU) of a particular preparation stimulated a 19*S* antibody response without a detectable 7*S* response provided a means of testing this possibility (Fig. 2). One group of five guinea pigs was injected on two occasions 1 month apart with 1 to 3 $\times$ 10^9 PFU of ϕX. The first and second responses were both composed of 19*S* antibody molecules without detectable 7*S* antibody, and there was no significant difference between the magnitude of the two responses. In contrast, a second group of five animals that received 3 to 6 $\times$ 10^9 PFU, which stimulated production of small amounts of 7*S* antibody at 4 weeks, showed a second antibody response after injection of 3 to 6 $\times$ 10^9 PFU in which the predominant antibody was 7*S*, the peak titer of 7*S* was reached earlier, and the magnitude of the response was considerably greater than in the first 7*S* response. Similar results were obtained in additional experiments with several dozen animals. It is unlikely that these results can be explained by a quantitative deficiency in the 19*S* response, since in the group that did not develop 7*S* immunological memory 19*S* precipitating antibody to ϕX was demonstrated by Preer double-diffusion in agar (*37*) in the majority of serums tested, indicating that a minimum of several micrograms of antibody protein per milliliter was present. Also, the primary 19*S* response of several animals that did not develop immunological memory was greater than the primary 19*S* response of other guinea pigs that did show immunological memory for 7*S* antibody. These findings suggest that in contrast to that of 7*S* antibody the 19*S* antibody mechanism does not usually develop a persisting immunological memory. Similar results have been obtained by Svehag and Mandel (*38*) with polio virus as antigen in rabbits. Exceptions to this pattern will be discussed subsequently.

It has been known since 1909 (*39*) that mixing excess amounts of specific antibody with antigen can diminish the capacity of antigen to stimulate antibody formation. Several years ago, in studies performed with J. Baumann, it was shown that passively administered diphtheria antitoxin can effectively inhibit the primary antitoxin response when administered as long as 5 days after immunization with toxoid in adjuvant (*40*). The secondary antitoxin response can also be inhibited, but to a lesser extent, by such passively administered antitoxin 4 days after "booster" injection (*41*). These observations led to the suggestion that antibody formation might function in part as a "feedback" type of mechanism. Presumably, during an immune response, the product of the response, specific antibody, can combine with and divert antigen that otherwise would stimulate further antibody formation. The above experiments, however, were performed with large amounts of passively administered hyperimmune serum. It was not clear whether antibody formed early in immunization could also inhibit antibody formation.

The results of studies, performed with M. S. Finkelstein, on the inhibition of formation of antibody to ϕX by passively administered antibody are as follows. "Late" immune antiserum to ϕX containing predominantly 7*S* antibody can inhibit the primary 7*S* antibody response even when injected 48 to 72 hours after an initial injection of antigen (ϕX) at the time that 19*S* antibody synthesis is in an exponential phase. Moreover, such treatment with antiserum can prevent the development of 7*S* immunological memory. In contrast, early immune serum the antibody of which is predominantly γ_{1M} (19*S*) is strikingly less effective in inhibiting antibody formation. These results taken together with the previous studies suggest that serum antibody can prevent hyperimmunization. Only as immunization proceeds, do antibody molecules appear with the capacity to inhibit antibody formation probably because of increasing binding affinity for antigen (*31*). Such a mechanism would prevent unlimited hyperimmunization to antigens that are continually presented to the circulation for long periods of time—a situation that probably occurs in chronic diseases caused by persisting infective agents, for example, tuberculosis, syphilis, helminth infections, and others.

The Role of Antigen in the Regulation of Immune Functions

The role of antigen in determining the specificity of the resultant γ-globulin antibody response is acknowledged to be the central question of im-

munology. It is not surprising, therefore, that other immune functions affected by antigen have received little attention, with the exception, perhaps, of immunological tolerance.

Although there have been numerous studies of the fate of labeled antigen in organs and cells (*42*), there has been no report of labeled antigen within antibody-forming cells. Thus, it has been possible to hypothesize that the presence of antigen is not essential for the continued synthesis of antibody (*43*) (selective theories), or that it is essential (*44*) (instructive theories).

The short duration and abrupt cessation of 19*S* antibody formation suggested the depletion of an essential factor, perhaps antigen. To test this possibility, guinea pigs immunized with ϕX received a second injection of specific antigen at the time that 19*S* antibody synthesis had virtually stopped.

Figure 3 shows the response of two groups of three animals each in such an experiment. As can be seen, a second injection of ϕX on day 9 can again stimulate 19*S* antibody synthesis. The prompt increase of serum antibody concentration after the second injection indicates synthesis by the same population of cells which had previously formed antibody. If a new population of cells had responded, there would have been a delay of approximately 1 week before antibody formation was sufficient to increase noticeably the existing high level of serum antibody. The stimulation by the second injection was immunologically specific since no such effect occurred in the control group which received T_2 bacteriophage on day 9. Moreover, in the controls, titers of antibody to T_2 5 days after the injection of T_2 were of the expected low values, thus excluding an alternative possibility that a nonspecific change in the lymphoid organs of immunized animals had occurred which allowed previously uncommitted cells to give an accelerated antibody response to the second injection of ϕX.

This experiment suggests therefore that antigen must persist for the continued synthesis of 19*S* antibody and that depletion of antigen can terminate such synthesis. Our results give no indication, of course, whether antigen must persist inside antibody-producing cells or need only interact with the cell surface or with other cells that release immunogenic factors.

In addition to the duration of 19*S* antibody synthesis, the dose of ϕX can affect several other aspects of the immune response. Table 1 lists these other functions and the doses of a particular ϕX preparation which affect the corresponding function (*45*). It was also observed that 5×10^{11} PFU of ϕX injected over a period of 5 days failed to induce tolerance in newborn rabbits, but studies by others (*46*) with different antigens indicate that a critical concentration of antigen exists above which immunological unresponsiveness is induced.

These observations indicate that there are critical levels of antigen which can control the appearance or relative rate of formation of different classes of antibody or both.

The nature of antigen is also important in the regulation of immune functions. Although certain antigens

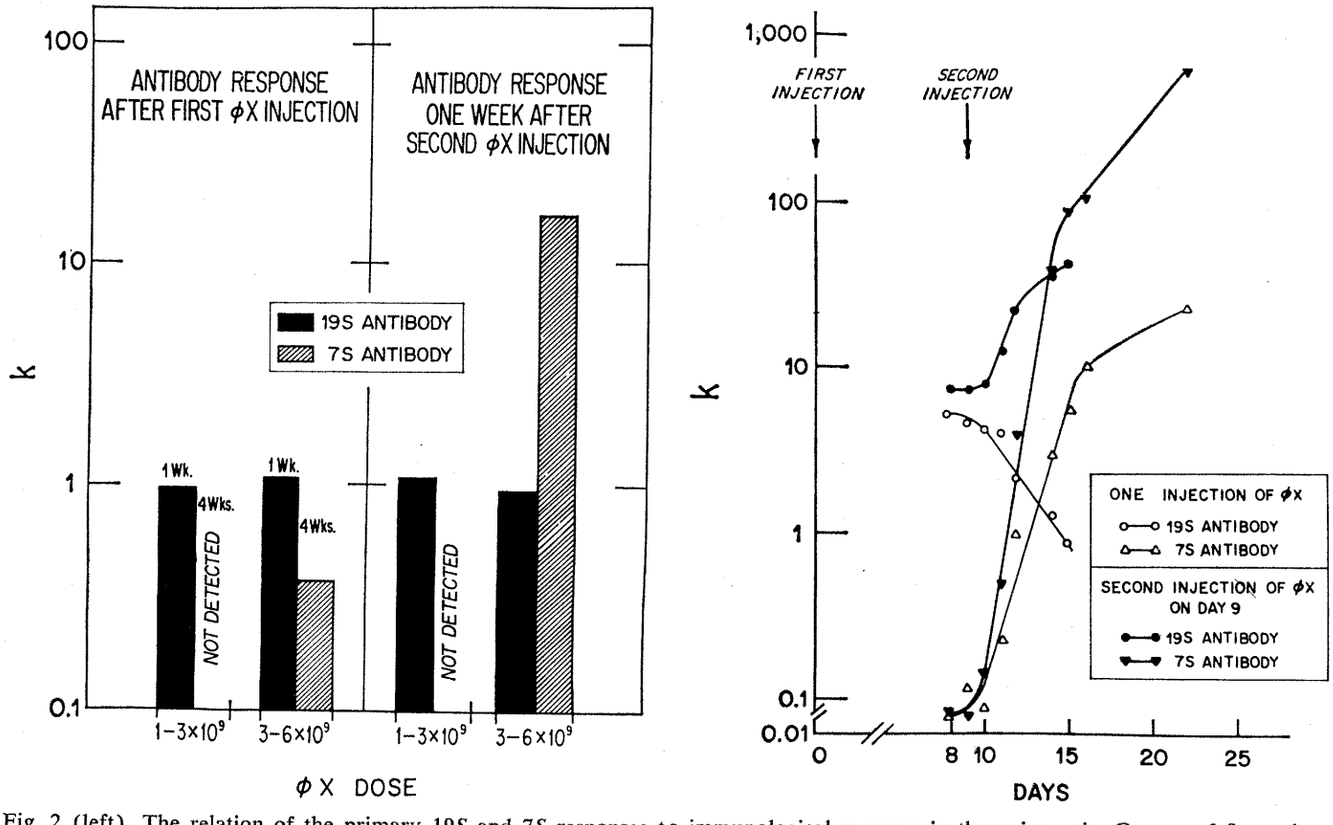

Fig. 2 (left). The relation of the primary 19*S* and 7*S* responses to immunological memory in the guinea pig. Groups of five guinea pigs each were injected twice at an interval of 1 month with either 1 to 3 $\times$ 10^9 PFU or 3 to 6 $\times$ 10^9 PFU of ϕX. Fig. 3 (right). The effect of a second injection of ϕX on day 9 on the primary 19*S* and 7*S* responses in the guinea pig. The average titers of antiserum to ϕX of two groups of three animals each are shown. All animals received 5 $\times$ 10^9 PFU of ϕX; one group was reinjected on day 9 with 10^{11} PFU of ϕX, and the other with 10^{10} PFU of T_2.

stimulate an easily detectable antibody response after a single injection of a small amount of antigen, certain other antigens require repeated injections of larger amounts, or the use of adjuvants in order to induce a quantitatively similar response. This difference is magnified in a striking manner, however, by immunization studies with diphtheria toxoid and ϕX in newborn humans (*28*) or sheep embryos (*27*) (Table 2). In newborn humans, formation of antibody to ϕX is demonstrable by 48 hours, and precipitating antibody is detectable as early as 1 week, in contrast to approximately 1 month for detection of diphtheria antitoxin (*47*). Antibody to ϕX is demonstrable in fetal sheep immunized at 35 days of age and bled 10 days later, and several hundred micrograms of antibody can be found if the interval between immunization and bleeding is increased to 30–70 days. In contrast, in toxoid-immunized embryos, antitoxin cannot be detected throughout the entire period of gestation (150 days) regardless of the method of antibody assay or whether Freund's adjuvant is included in the immunizing injection.

Studies comparing the kinetics of antibody formation to bacteriophages T_2 and ϕX in rabbits and guinea pigs (*48*) revealed that 7*S* antibody to T_2 was usually detectable on day 3 compared to day 7 or 8 for 7*S* antibody to ϕX and that the 19*S* antibody response to T_2 usually persisted longer than that to ϕX.

These differences were not unexpected because although there are a considerable number of different antigens reported to stimulate relatively short-lived 19*S* antibody formation, it is also known that *Salmonella* "O" (*18*) and human red blood cells (*49*) can both stimulate predominantly 19*S* antibody for long periods of time, even years; moreover, the red blood cells supposedly stimulate long-lived 19*S* immunological memory. An unexpected finding, however, was that a second preparation of ϕX stimulated detectable 7*S* antibody in both rabbits and guinea pigs as early as day 4 and formation of 19*S* antibody lasted several days longer than usual. Thus in addition to inherent structural differences between molecules, other factors, such as aggregation, may influence the chronology of the sequential appearance of these two classes of antibodies and presumably the duration of 19*S* immunological memory. Nossal *et al.* (*50*) have reported recently that *Salmonella* flagellin monomer in contrast to the polymer stimulated 7*S* antibody formation without a prior phase of 19*S* antibody formation, suggesting that stimulation of the 19*S* system may be favored by particulate antigens.

These considerations indicate the difficulty in generalizations concerning antibody formation based upon defined experimental conditions. Depending upon the dose, the nature and physical state of the antigen, the species, the age of the host, and a variety of other factors, different immunization patterns can be obtained.

Discussion

The mechanisms by which dose or nature of antigen regulates the aforementioned immunologic functions are not known. The functions affected by the nature of the antigen probably depend on characteristics of the entire molecule rather than on the antigenic determinants themselves. This is well illustrated by the slight but definite differences between two preparations of ϕX which have similar immunologic specificity. It is difficult to explain how these characteristics can so strikingly affect immunologic functions without postulating that catabolism of antigen is an essential step for primary immunization. It is known that the majority of circulating antigenic molecules are removed by cells of the reticuloendothelial system (*42*), but these cells have not been shown to participate directly in the formation of antibody. Studies by Haurowitz (*51*) and Garvey and Campbell (*52*) indicate an association of antigenic fragments with the ribosomal fraction of such cells. The significance of these two observations may have been elucidated by studies (*3, 53*) showing that T_2 bacteriophage must first be processed by macrophages in order to stimulate the formation of specific antibody by normal lymphoid cells in vitro. Within these macrophages, digested T_2 phage becomes associated with RNA, and treatment of the extract with ribonuclease inhibits its capacity to stimulate the normal lymph node cells. Consistent with this result are the studies suggesting that amino acid polymers must be metabolized by the host in order to be antigenic (*54*). If catabolism of the immunizing material does occur, however, tertiary structure of the fragments can be maintained, since rabbit antibodies to intact polio virus, to virus protein coat without its RNA core, and to subunits of the protein coat have different specificities with little or no cross reactivity (*55*).

Dr. G. Weissmann and I have followed the fate of ϕX and T_2 in the liver, spleen, and lymph nodes of normal rabbits and guinea pigs. These studies confirm and extend those of Franzl (*56*) and indicate that the bacteriophages become associated with and are degraded in the large granule fraction (containing lysosomes and mitochondria) of the cell. Plaque-forming ϕX can be found localized in the large granule fraction 1 hour after injection of phage, and 48 hours after phage injection this fraction, which no longer contains plaque-forming material, can stimulate formation of antibody to ϕX upon injection into normal animals. Plaque-forming bacteriophage can be released from the large granule fraction by treatment with a membrane disruptor such as lysolecithin, indicating that the phage is contained in membrane-bound structures, presumably lysosomes. The ϕX was also found in the ribosomal fraction of the cell, but efforts to compare the kinetics of phage association with the large granule and ribosomal fractions were hampered because of the similarity of sedimentability between ϕX and ribosomes.

It can be proposed, therefore, that after entrance into the circulation or lymph, antigen enters cells of the reticuloendothelial system, localizes in lysosomes, and is usually catabolized by lysosomal enzymes into smaller fragments; these fragments become associated with ribosomes; and the antigen (or RNA or a complex of both) is then transferred to the lymphocyte precursor of the plasma cell. This does not account for the fate of antigen within or on the surface of the antibody-producing cell itself, nor does it signify how the amount present and the nature of degraded antigen can affect functions dependent upon DNA —such as differentiation and replication of lymphoid cells.

There are other important unanswered questions relating to the regulation and heterogeneity of the immune response.

It is not definitely known whether one cell usually produces more than

one class of antibody, that is, whether there is a differentiation process within a single cell or cell line. Nossal (*57*) has recently shown that there are some cells that can simultaneously produce both 19*S* and 7*S* antibody to flagellar antigen of *Salmonella*. The question remains, however, whether such dual capacity is only expressed in a small minority of the antibody-producing cells analogous to the small number of cells simultaneously producing antibodies of two different specificities in animals immunized to two antigens (*58*).

The question of the significance of the heterogeneity of the immune response cannot be answered satisfactorily at present. However, during immunization, there is a progressive increase in the binding affinity of 7*S* antibody molecules that appear in the circulation (*31*). The difference of the binding affinity between early and late immune serum may be as great as 10,000-fold. Thus, during immunization, there is a progressive change in the quality of 7*S* antibody molecules produced resulting in an increased efficiency in their capacity to bind to specific antigen. Presumably, antigen is acting as a stimulus to proliferation, and possibly also as a selective agent because of cellular differences in efficiency of antigen capture. Grey (*32*) in a study of the 19*S* antibody response in turtles has not found such a change in binding affinity. Moreover, 19*S* in contrast to 7*S* diphtheria antitoxin does not neutralize diphtheria toxin in vivo (*59*); 19*S* antibody to red blood cells, however, can be extremely efficient in binding to red blood cells and causing hemolysis or agglutination (*60*). Thus, the γ_{1M}-system might be regarded as a first line of defense, capable of prompt production of large numbers of antibody molecules which can readily "coat" particulate antigens such as bacteria but which are relatively inefficient in their capacity to bind to soluble antigens such as toxins, and in which the cells do not take part in long-lived synthesis, in the improvement in the quality of antibody produced, or in the development of immunological memory. In contrast, the 7*S* system may be initially represented by a small population of cells not capable of an immediate vigorous response, but eventually able to synthesize more efficient antibody molecules for long periods of time, and to participate in the development of a persisting immunological memory.

Another clue to the possible significance of an immune system composed of different classes of immune globulins is provided by the recent observation that γ_{1A}-globulin alone is selectively secreted by the parotid gland (*61*), and that this class of γ-globulin, and not the other immunoglobulins, binds to skin, smooth muscle, and mucous membranes (*12*). "Bound" γ_{1A}-antibody after interaction with antigen can cause release of histamine and other pharmacologic agents at the local site of interaction (*62*). The significance for immunity of histamine release is not yet known, but it is possible that these unusual biological attributes of γ_{1A}-globulin may play a role in defending exposed body cavities to surface penetration by infective agents. In any event, it is probable that large metazoan species, such as the human, face a variety of problems in defending themselves against different aggressor microorganisms. In addition, the immune system may also have an important role in homeostasis, possibly in defending against neoplasia as a result of somatic mutation (*63, 43*). These varied challenges might best be met by a multicomponent immune system in which each component is specialized to perform a particular function at a particular time.

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Empiricism in Latter-day Behavioral Science

Developments in this field, as in other sciences, invite critical review and corrective revision.

V. Edwin Bixenstine

The mood of behavioral science today is sometimes difficult to assess. There is a rising flow of research, as reported in journals, at regional and national meetings, and at a growing number of invitational conferences. We can only be impressed by the energy and the enthusiasm evinced by the participants. A great deal is being done.

On the other hand, there is an undercurrent of perplexity and doubt. While more persons register at our conventions than ever before, attendance at section meetings for the presentation of papers is embarrassingly thin, and little serious attention is given to the research reported. It is a safe bet that there is now a high inverse relationship between mass of reported works and the attention each receives.

There is a general feeling that we behavioral scientists have less confidence today about our grasp of the field than we had 20 years ago. A reviewer recently commented that the days of the grand theory, à la C. L. Hull or E. C. Tolman, are gone. We now bite off small chunks in specialized areas. Some groupings of behavioral scientists are characterized by inability or lack of desire to communicate with any but the insiders. Sometimes these groupings are established as a result of exclusion; for example, J. B. Rhine and his extrasensory perception group inaugurated a journal because editors refused to publish their work. On the other hand, B. F. Skinner's journal has the mark of aristocracy; it is a product of selective inbreeding.

If our work is often unattractive to all but a few of us, we find little to console us in the recurrently critical judgment of the laity. Public acceptance is far from crucial as regards the intrinsic merit of a research project. On the other hand, can we be sure that lack of lay enthusiasm for projects dear to the hearts of behavioral scientists is always a function of lack of public understanding? Might it be that we have psychic investments in our topics and methods quite different from the need to know, understand, and relate? I think that we do.

Events conspire today to impel the scientist into certain forms of research activity. We have had so few "breakthroughs" in behavioral science that we no longer approach research with the faint but uplifting hope that *this* time an important, vital insight will result. Number of published works has more to do with status than the importance of the work has. Journal editors have gradually altered their publication policy: reports must be brief, nontheoretical, and on empirical research that is simple both in design and results. Monetary support from granting agencies is likely to go to someone with a "program." This sounds fine, since it encourages systematic development of an inquiry, but inevitably it also means investing in certain kinds of apparatus and in certain procedural and methodological tools which tend to fix the approach and reduce receptivity to new possibilities. No one receives support who says, in effect, I will study X, using procedures a, b, or c, and if my interest in X wanes, I will study Z, using procedures d, e, or f.

The value placed on publication, the editorial policies of journals, and the impact of granting institutions converge in effecting what I call "production-line research." This is research which revolves around a gimmick—a fixed procedural tool or method with which the researcher produces a series of studies, using first one set of variables and then another, systematically plotting some "behavioral space" as defined by the operational coordinates used.

The Special Impact of Skinner

Woven through these developments in the practice of our science is a complementary philosophy and rationale. Disturbed as some of us may be about the way behavioral science is practiced, what is more disturbing is the fact that many others approve, and often talk as if we were approaching the ideal practice of our science. I believe this remarkable complacency can be traced largely to the impact of B. F. Skinner. I suspect that Skinner will emerge historically as one of the most influential behavioral scientists of the mid-20th century. His writings are clear, scholarly, persuasive. He has, as a teacher, great capacity to inspire a loyal following. Add to these attributes

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