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CURRENT PROBLEMS IN RESEARCH

Genes and Antibodies

Do antigens bear instructions for antibody specificity or do they select cell lines that arise by mutation?

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An antibody is a specific globulin which appears in the serum of an animal after the introduction of a foreign substance, an antigen (*1*). Each of the many globulins is specified by its reaction with a particular antigen (*2*). Our present concern is to formulate a plausible mechanism for the role of the antigen in evoking large amounts of a specific complementary globulin. An important element of any theory of antibody formation is its interpretation of self-recognition, the means by which an organism discriminates its own constituents from the foreign substances which are valid stimuli of the immune response.

Recent speculation about antibody formation (*3–8*) has been dominated by instructive theories which suppose that the antigen conveys the instructions for the specificity of the globulin synthesized under its governance. Elective theories date from Ehrlich (*9*) and have been revived principally by Jerne (*10*), Talmage (*2, 11*), and Burnet (*12*). These postulate that the information required to synthesize a given antibody is already inherent in the organism before the antigenic stimulus is received, and the stimulus then functions to stimulate that mechanism electively. Jerne had proposed an elective transport of antibody-forming templates to functioning sites; Talmage and Burnet have explicitly proposed an elective function based on cellular selection. The details which distinguish the various proposals are pointed out in the following discussion.

Immunology does not suffer from a lack of experimental data, but still some of the most elementary questions are undecided, and it is not yet possible to choose between instructive and elective theories. However, the latter have had so little expression in the past few decades that a detailed exposition may serve a useful function, if only as a target for experimental attack. This article is an attempt to formulate an elective theory on the basis of genetic doctrines developed in studies of microbial populations.

Of the nine propositions given here, only number 5 is central to the elective theory. The first four are special postulates chosen as an extreme but self-consistent set; however, they might well be subject to denial or modification without impairing the validity of the elective approach. The last four propositions are stated to account for the general features of antibody formation in cellular terms and may be equally applicable to instructive and elective theories. If this theory can be defended, and I know of no fatal refutation of it, then clearly elective theories of antibody formation perhaps less doctrinaire in detail should have a place in further experimental design, each proposition being evaluated on its own merits. I am particularly indebted to Burnet (*13*) for this formulation, but Burnet should not be held responsible for some elaborations on his original proposal, especially in propositions 1 through 4. A connected statement of the nine propositions is given in Table 1, and each one is discussed in detail in the following sections.

Antibody Globulin

A1. *The stereospecific segment of each antibody globulin is determined by a unique sequence of amino acids.*

This assertion contradicts the more popular notion, and the usual basis of instructive hypotheses, of a uniform sequence subject to differential folding. The chemical evidence is far from decisive. For example, Karush (*14*) rejects this proposition not on analytical evidence but on the cogent argument that miscellaneous antigenic compounds can scarcely convey instructions for sequence. But if instructive-sequence is implausible, this perhaps argues against instruction rather than differential sequence. Karush has also demonstrated the remarkable stability of antibody through cycles of exposure to denaturing concentrations of urea. He attributes the structural continuity to stabilizing disulfide linkages, but determinant amino acid sequences may also be involved.

Elective antibody formation is of course equally compatible with sequence or folding. In such a theory, the mechanism of assembly does not have to be specified, so long as the product (the prospective antibody) recognizes—that is, reacts with—the antigen. Differential sequence is proposed (i) to stress the ambiguity of present evidence and (ii) as being more closely analogous to current conceptions of genically controlled specificity of other proteins (*15*).

The direct analysis of antibody structure by physicochemical methods has been equivocal. The fractionation of globulins by partition chromatography (*16*) might be interpreted by differential exposure of phenolic, amino, and carboxyl groups rather than differences in essential composition. Characterization of amino acid composition has given sharply different results with rabbit globulins, on the one hand, and equine and human globulins, on the other. Rabbit globulins, including various antibodies, apparently have a uniform N-terminal sequence, so far identified for five residues as (*17*):

Alanine-leucine-valine-aspartic-glutamyl

Various antibodies were, furthermore, indistinguishable in over-all composition (*18*). Any chemical differences would then have to attach to a central, differential segment. This possibility is made more tangible by Porter's recent finding (*19*) that rabbit antibody globulin could be split by crystalline papain into three fragments. One of these was crystallizable (and presumably homogeneous), devoid of *antibody* activity, but equivalent as an *antigen* to the intact globulin. The remaining fractions were more heterogeneous and retained the antigen-combining specificity of the intact antibody. As these fractions may well correspond to the differential segments, their

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Table 1. Nine propositions.

A1. The stereospecific segment of each antibody globulin is determined by a unique sequence of amino acids.

A2. The cell making a given antibody has a correspondingly unique sequence of nucleotides in a segment of its chromosomal DNA: its "gene for globulin synthesis."

A3. The genic diversity of the precursors of antibody-forming cells arises from a high rate of spontaneous mutation during their lifelong proliferation.

A4. This hypermutability consists of the random assembly of the DNA of the globulin gene during certain stages of cellular proliferation.

A5. Each cell, as it begins to mature, spontaneously produces small amounts of the antibody corresponding to its own genotype.

A6. The immature antibody-forming cell is hypersensitive to an antigen-antibody combination: it will be suppressed if it encounters the homologous antigen at this time.

A7. The mature antibody-forming cell is reactive to an antigen-antibody combination: it will be stimulated if it first encounters the homologous antigen at this time. The stimulation comprises the acceleration of protein synthesis and the cytological maturation which mark a "plasma cell."

A8. Mature cells proliferate extensively under antigenic stimulation but are genetically stable and therefore generate large clones genotypically preadapted to produce the homologous antibody.

A9. These clones tend to persist after the disappearance of the antigen, retaining their capacity to react promptly to its later reintroduction.

further immunological and chemical analysis will be of extraordinary interest.

In contrast to the uniformity of rabbit globulins, normal and antibody globulins of horse serum proved to be grossly heterogeneous but equally so, a wide variety of N-terminal groups being found in all preparations (*20*). This merely confirms the concept of the plurality of antibodies evoked by a given antigen, which have in common only the general properties of normal gamma globulins and the capacity of reacting with the evoking antigen. The globulins of man, and in particular the characteristic globulins produced by different patients suffering from multiple myeloma, are likewise recognizably different, inter se, in amino acid composition (*21*).

Gene for Globulin Synthesis

A2. *The cell making a given antibody has a correspondingly unique sequence of nucleotides in a segment of its chromosomal DNA: its "gene for globulin synthesis."*

This postulate follows plausibly from proposition A1, and would trace antibody-forming specificity to the same source as is imputed to other specific proteins. As the most deterministic of genetic hypotheses, it should be the most vulnerable to experimental test. For example, a single diploid cell should be capable of *at most* two potentialities for antibody formation, one for each chromosome.

In tests of single antibody-forming cells from rats *simultaneously* immunized against two *Salmonella* serotypes, Nossal and I (*22*) could find only monospecific cells producing one or the other antiflagellin. Coons (*23*) and White (*24*) have reached a similar conclusion in applications of fluorescent labeling technique. However, Cohn and Lennox (*25*) have convincing evidence for some bispecific antibody-forming cells in rabbits *serially* immunized against two bacteriophages. Experiments pertinent to the possibility of a single cell's carrying more than two antibody-forming specificities remain to be done (*26*).

The chromosomal localization of antibody-forming specificity is uncoupled from its elective origin in proposals (*7, 8, 27*) that an antigen induces a mutation in a gene for globulin synthesis, though not necessarily involving a new nucleotide sequence.

Multiple specificity would stand against a simple chromosomal basis for antibody formation (*28*), leaving two alternative possibilities: (i) replicate chromosomal genes or (ii) extrachromosomal particles such as microsomes. These might best be disentangled by some technique of genetic recombination.

The differentiation of microsomes must be implicit in any current statement of a theory of antibody formation that recognizes their central role of protein synthesis. The main issue is whether or not their specificity is dependent on that of the chromosomal DNA. Autonomy of microsomes, in contradiction to proposition A2, is implicit in most instructive theories, the microsome carrying either the original or a copy of the antigenic message. On the other hand, a powerful elective theory is generated by substituting the term *microsomal RNA* for the terms *chromosomal DNA* and *gene* in the various propositions. Since a single cell may have millions of microsomes, this theory would allow for any imaginable multiplicity of antibody-forming information in a single cell. If the potential variety of this information approaches that of the total antibody response, further instructions in an antigenic input would become moot. In addition, the complexities of selection of cellular populations would be compounded by those of microsomal populations within each cell. These degrees of freedom which blur the distinction between microsomal instruction and election favor the utility of the chromosomal hypothesis as a more accessible target for experimental attack.

Genic Diversity of Precursor Cells

A3. *The genic diversity of the precursors of antibody-forming cells arises from a high rate of spontaneous mutation during their lifelong proliferation.*

Three elements of this statement should be emphasized: (i) that antibody-forming cells are specialized, (ii) that their diversity arises from some random process, and (iii) that the diversification of these cells continues, in company with their proliferation, throughout the life of the animal.

Item (i) and its justification by various experiments have already been discussed as an aspect of proposition A2. Talmage (*2*) also stresses the specialization of antibody-forming cells by referring to their progressive *differentiation*. This is entirely consistent with propositions A3 and A4, which then postulate a specific mechanism of cellular differentiation, in this case, gene mutation. If, on Talmage's model, fully differentiated cells are ultimately left with no more than one antibody-forming specificity per chromosome, the general consequences will be the same whether this final state represents the unique activation of one among innumerable chromosomal loci (see *27*) or the evolution of one among innumerable specific alleles at a given locus. Once again, the final resort for decision may have to be a recombinational technique.

If the discrepancy between the experiments of Nossal and Lederberg (*22*) and those of Cohn and Lennox (*25*), as dis-

cussed under proposition A2, is real and depends on the timing of immunization, it may furnish strong support for (ii), the random origin of antibody-forming specificity. If antibody-forming cells can have two (or any small number of) specificities randomly derived, only a negligible proportion will have just the two being tested for. This would correspond to the case of simultaneous immunization with the two test antigens. If, however, a population of cells carrying one specificity is selected for, followed by selection for a second specificity among all available cells, this is the case of serial immunization and is precisely the method one would predict to obtain a clone "heterozygous" for two mutant alleles. Simultaneous versus serial immunization would be analogous to the suppression versus selection of bacterial mutants resistant to two antibiotics (*29*). Further experiments are needed to exclude more trivial reasons for the scarcity of bispecific antiflagellin-forming cells.

Item (iii) diverges from Burnet's proposal that the "randomization" of antibody-forming cells is confined to *perinatal* life, thereby generating a set of then stable clones corresponding to the antibody-forming potentiality of the animal. These clones would then be irreplaceable if lost either by random drift or as a consequence of premature exposure to the corresponding antigen. The arguments against Burnet's proposal are by no means decisive; however, the correspondence between cells and antibodies is made more difficult by having to maintain each clone at a sufficient population size to compensate for loss by random drift. Further, the recurrence of antibody-forming specificity is supported by experiments showing the decay of immune tolerance in the absence of the corresponding antigen (*30*; see comment on proposition A6). Since immune reactivity in these experiments may return during adult life, susceptibility to the induction and maintenance of tolerance by the timely introduction of the antigen may have only a coincidental relationship to the immunological incompetence of the newborn animal.

Hypermutability

A4. *This hypermutability consists of the random assembly of the DNA of the "globulin gene" during certain stages of cellular proliferation.*

This *ad hoc* proposal is doubtless the least defensible of the propositions, and certainly the furthest removed from experimental observation. It is stated to illustrate that accurate replication rather than mutability is the more remarkable phenomenon, whatever the detailed mechanism for the variation. If, as has been suggested, many nucleotide triplets are *non*sensical (*31*), the triplets rather than single nucleotides would have to be posed as the unit of assembly in this case.

To carry this speculation one step further, *heterochromatin* has been proposed to be, on the one hand, a random sequence, and, on the other hand, a dissynchronously assembled segment of the genome (*32*). If both views are correct, proposition A4 might be restated: "the globulin gene is heterochromatic during certain stages of cellular proliferation" (becoming by implication, euchromatic in the mature stages of propositions A8 and A9).

For the theory of microsomal election it might be postulated that globulinogenic microsomes are initially fabricated as faulty replicas of the globulin gene, but are then capable of exact, autonomous replication.

Pending more exact knowledge and agreement of opinion on the morphogenetic relationships of antibody-forming cells, the term *certain stages* cannot be improved upon. On the other hand, as is shown under proposition A8, a model might be constructed even on the basis of a constant but high mutation rate of all antibody-forming cells.

Further insight into the mechanism of cellular diversity in antibody formation may be won by studies on the genetic control of reactivity to various antigens in inbred animals (*33*); two cautions, however, must be stated: (i) for effects on the transport of particles of different size, and (ii) for effects from cross-reactions with gene-controlled constituents evoking autotolerance.

Spontaneous Production of Antibody

A5. *Each cell, as it begins to mature, spontaneously produces small amounts of the antibody corresponding to its own genotype.*

Note the implication that antibody is formed prior to the introduction of the antigen into the antibody-forming cell.

The function of spontaneous antibody is to mark those cells preadapted to react with a given antigen, either to suppress these cells for the induction of immune tolerance (proposition A6) or to excite them to massive antibody formation (proposition A7). Therefore, the antigen need participate in no type of specific reaction with cell constituents other than antibody itself, the one type of reaction available to chemically diverse antigens that requires no further special pleading. There is no agreement whether the reactive globulins found in the serum of untreated animals are produced spontaneously or by casual exposure to cross-reacting antigens (see *2*). Accordingly, the spontaneous antibody postulated in proposition A5 may or may not be produced in the quantity and form needed for it to be liberated and detected in the serum. The nonspecific fragment of antibody-globulin described by Porter raises the possibility that the same *determinant* segment may be coupled either to a diffusible or to a cell-bound residue, the latter corresponding to various aspects of cellular immunity, including the suppression or excitation of antibody-forming cells by reactions with the corresponding antigen.

Induction of Immune Tolerance

A6. *The immature antibody-forming cell is hypersensitive to an antigen-antibody combination: it will be suppressed if it encounters the homologous antigen at this time.*

This is the first of four propositions which bear less on the source of antibody-forming specificity than on its subsequent expression in terms of cellular behavior. These propositions are therefore equally applicable to instructive theories.

The duality of reactions of antigens with antibody-forming cells is simply a restatement of the experimental observations of tolerance versus immunity (*34*). It seems plain that every cell of the antibody-forming system must be marked to inhibit its reactivity both to the autologous antigens of the same animal and extraneous antigens introduced and maintained from a suitably early time of development. In the light of current evidence for the persistence of antigenic molecules (*5, 6*) and for the loss of tolerance when a given antigen has dissipated (*30*) there are no more plausible candidates for the self-markers then the antigens themselves. The distinction between the function of an antigen as inhibitor (self-marker) or as inducer of antibody formation is then the time when the antigen is introduced into the potential antibody forming cell. We may profitably define maturity in terms of

the progression of the cell from sensitivity towards reactivity.

The suppression of this process of maturation is a sufficient attribute to account for tolerance, and this need not involve so drastic an event as the destruction of the cell. However, the elective hypothesis proposes that only a limited number of cells will spontaneously react with a given antigen, so that their destruction by premature reaction can safely be invoked as the means of their suppression. It may be hoped that presently documented phenomena of cellular hypersensitivity may furnish a precedent for cellular destruction by such reactions. The cytotoxicity of the antigen to hypersensitive cells is still controversial even in the historical case of tuberculin sensitivity (*35*). However, the destruction of invading *lymphocytes* of the host in the course of rejection of a sensitizing homograft (*36*) supports the speculation of some role of cellular destruction of immature antibody-forming cells in the induction of tolerance.

The nature of immaturity remains open to question. It might reflect the morphogenetic status of the antibody-forming cell—for example, sensitive lymphocyte → reactive plasma cell (*37*), some particular composition of immature sensitizing antibody, or merely a very low level of antibody so that complexes are formed in which antigen is in excess.

Finally, one additional hint of an implication of hypersensitivity in the early stages of the antibody response: the transient skin sensitivity of delayed type (and transferable by cells) appearing in the course of immunization, as observed by several workers (*38*). If these skin reactions reflect the destruction of some antibody-forming cells, it would speak for some overlapping or reversibility of the two stages of maturation.

The implications of proposition A6 in the elective theory may be summarized as follows: If an antigen is introduced prior to the maturation of any antibody-forming cell, the hypersensitivity of such cells, while still immature, to an antigen-antibody reaction will eliminate specific cell types as they arise by mutation, thereby inducing apparent tolerance to that antigen. After the dissipation of the antigen, reactivity should return as soon as one new mutant cell has arisen and matured. As a further hopeful prediction, it should be possible to induce tolerance in clones of antibody-forming cells from adult animals by exposing a sufficiently small number of initials to a given antigen.

Excitation of Massive Antibody Formation

A7. *The mature antibody-forming cell is reactive to an antigen-antibody combination: it will be stimulated if it first encounters the homologous antigen at this time. The stimulation comprises an acceleration of protein synthesis and the cytological maturation which mark a "plasma cell."*

These principles of the cellular response to *secondary* antigenic stimulation are widely accepted and are readily transposed to the *primary* response on the elective hypothesis whereby some cells have spontaneously initiated antibody formation according to proposition A5.

Proliferation of Mature Cells

A8. *Mature cells proliferate extensively under antigenic stimulation but are genetically stable and therefore generate large clones genotypically preadapted to produce the homologous antibody.*

This proposition takes explicit account of the secondary response, the magnitude of which is a measure of the increase in number of reactive cells (*26*). However, the antigen *need* play no direct part in the stabilization of antibody-forming genotype which might accompany the determinate maturation of the cell whether or not it is stimulated. In fact, it may be possible to dispense with the postulate that mature cells are less mutable by adopting a mutation rate which is an effective compromise: to furnish a variety of genotypes for the primary response while selected genotypes may still expand for the secondary response. For example, by mutation of one daughter chromosome per ten cell divisions, on the average, after ten generations about 600 chromosomes of the same type would have been produced, together with 100 new genotypes distributed among the other 400 or so cells. Selection must then compensate for the mutational drift if a given clone is to be maintained.

Persistence of Clones

A9. *These clones tend to persist after the disappearance of the antigen, retaining their capacity to react promptly to its later reintroduction.*

This is a restatement of the possibly controversial phenomenon of lifelong immunity to viruses (*4, 5*). A substantial reservoir of immunological memory should be inherent from one cycle of expansion of a given clone. Its ultimate decay might be mitigated either by continued selection (that is, persistence of the antigen) stabilization of genotypes, or dormancy (to cell division or remutation, or both) on the part of a fraction of the clone.

Discussion

Each element of the theory just presented has some precedent in biological fact, but this is testimony of plausibility, not reality. As has already been pointed out, the most questionable proposition is A4, and it may be needlessly fanciful to forward a too explicit hypothesis of mutability for antibody formation when so little is known of its material basis anywhere.

Theories of antibody formation have, in the past, been deeply influenced by the physiology of inducible enzyme synthesis in bacteria. In particular, instructive theories for the role of the substrate in enzyme induction have encouraged the same speculation about antibody formation. This interpretation of enzyme induction, however, is weakened by the preadaptive occurrence of the enzymes, at a lower level, in uninduced bacteria (*39*).

One of the most attractive features of the elective theory is that it proposes no novel reactions: the only ones invoked here are (i) mutability of DNA; (ii) the role of DNA, presumably through RNA, as a code for amino acid sequence and (iii) the reaction between antibody and antigen, already known to have weighty consequences for cells in its proximity. The conceptual picture of enzyme induction would be equally simplified if the enzyme itself were the substrate-receptor. Clearly, susceptibility to enzymic action is not a necessary condition for a compound to be an inducer —for example, neolactose and thiomethylgalactoside for the β-D-galactosidase of *Escherichia coli* (*39, 40*), but formation of complexes with the enzyme may be. The picture is somewhat complicated by the intervention of specific transport systems for bringing the substrate into the cell (*40*).

Antibody formation is the one form of cellular differentiation which inherently requires the utmost plasticity, a problem for which the hypermutability of a patch of DNA may be a specially evolved solution. Other aspects of differentiation

may be more explicitly canalized under genotypic control. Nucleotide substitution might still play a role here by modifying the level of activity rather than the specificity of neighboring loci, and elective recognition of transient states spontaneously derived then remains as a formal, if farfetched, possibility for other morphogenetic inductions.

References and Notes

1. This definition excludes antibody-like substances such as the hemagglutinins found in normal human sera. These reagents do not, however, pose the problem of the mechanism of specific response which is the burden of this discussion.
2. Talmage, in this issue of *Science*, discusses various aspects of antibody specificity, including the number of antibodies, which may be exaggerated in current immunological thought. For the present discussion, however, this number is left open for experimental determination, for it would embarrass a theory of cellular selection only if it is large compared with the number of potential antibody-forming cells in the organism. To anticipate proposition A1, as few as five determinant amino acids would allow for $20^5 = 3{,}200{,}000$ types of antibody.
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23. A. H. Coons, *J. Cellular Comp. Physiol.* 50, Suppl. 1, 242 (1957).
24. R. G. White, *Nature* 182, 1383 (1958).
25. M. Cohn and E. S. Lennox, private communication.
26. An indirect measure of polyspecificity would be the total number of antibodies multiplied by the proportion of competent cells *initially* recruited to yield a particular species. Coons (7) has not attempted to count the antibody-forming cells in primary response, but his statements are compatible with an incidence of 10^{-5} to 10^{-3} of cells forming antialbumin in lymph nodes 4 days after inoculation. Nossal (*Brit. J. Exptl. Pathol.*, in press) found about 2 percent of yielding cells in a primary response after 7 days. These figures are subject to an unknown correction for the extent of proliferation in the interval after inoculation. They perhaps also raise the question whether all the yielding cells are indigenous to the lymph node, or whether circulating cells of appropriate type can be filtered by a node in which locally administered antigen has accumulated.
27. J. Schultz, *Science* 129, 937 (1959). Schultz makes an analogy between antibody formation and serotype determination in *Paramecium*, stressing the role of cytoplasmic feedback mechanisms in the *maintenance* of specificity.
28. A diploid cell should be heterozygous for at most two alleles at one locus, but strictly speaking, this is a restriction of genotype, not phenotype. A cell whose proximate ancestors had mutated through a series of different states might carry a phenotypic residue of information no longer represented in its chromosomes [see linear inheritance in transduction clones: B. A. D. Stocker, *J. Gen. Microbiol.* 15, 575 (1956); J. Lederberg, *Genetics* 41, 845 (1956)]. Pending tests on clones from single cells, bi- or polyspecificity of antibody-forming phenotype remains subject to this qualification.
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39. J. Lederberg, in *Enzymes: Units of Biological Structure and Function*, O. H. Gaebler, Ed. (Academic Press, New York, 1956), p. 161. A feeble attempt in this paper to homologize antibody formation with elective enzyme induction was hindered by an uncritical rejection of proposition A1 and by the want of a tangible cellular model such as Burnet and Talmage have since furnished.
40. J. Monod, *ibid.*, p. 7.

Addendum to "Genes and Antibodies"

(SCIENCE 129:1649-53, 1959)

With the help of some perspective and many discussions since this ms. was written (Nov. 1958), some obscurities in this presentation might now be clarified. The following comments are also represented in discussions elsewhere (SCIENCE, in press: "A view of genetics"; Ciba Foundation Symposia: Human Biochemical Genetics (Naples, May 1959); Cellular Aspects of Immunity (Royaumont, June 1959); Summary Comment Gatlinburg Symposium, J. Comp. Cell. Physiol.52, Suppl. 1:383-402, 1959).

A1. The dependence of the three-dimensional shape (folding) on the amino acid sequence should be stressed; more precisely (following S. Brenner) the indicated sequence as the polypeptide is formed determines the folding. We should not overlook the possibility, however, that DNA contains information not only for amino acid sequence but also for folding: viz., through interstitial punctuation or spacing among the (triplet?) codes for amino acids. The functional specificity of the protein will, of course, finally depend on the shape into which it has folded.

A2. The "gene for globulin synthesis" refers to the differential segment of the antibody molecule, not to the common segment. Grubb's Gm gene (Ciba, Naples) is a likely candidate for a gene for the common segment.

A2 and A3. The discrepancy between the findings of Coons, of Nossal, and of White respectively, contra those of Cohn and Lennox has not yet been resolved. The main difference in the experiments seems to be the duration of immunization, which was relatively short in the former, prolonged in the latter. This provokes the suggestion that bispecific cells may be sequential steps, for example, spontaneous mutations. Prolonged immunization may also allow for exchange of information among cells, e.g., phagocytosis with retention of the microsomes of the eaten cell, not to exclude other processes of cellular recombination.

A4. Undue prominence is given to this parenthesis on random assembly and heterochromation. It simply illustrates one of many possible ways to understand high mutation rates.

A5. "Each cell" should be read, "Each cell of the antibody-forming lineage."

A6. The importance of cytotoxicity of the antigen in hypersensitive states is being warmly debated, but there is no debate over the destruction of graft cells in the homograft reaction. For this, we picture a lethal encounter between an immune lymphocyte and an antigenic graft cell. A soluble antigen may destroy such cells indirectly, its combination with adherent antibody sensitizing them to destruction by other cells.

A8. That is, if an antigen stimulates a large issue from a particular cell it is not necessary to invoke the added element of genetic stabilization. The amplification of progeny would suffice to insure the retention and preeminence of that clone.

The RNA-microsomal hypothesis should be clarified. The essence of the theory, embodied in A5, is the preadaptive genotypic diversity of antibody-forming cells. If the mutation occurs in a typical gene, in chromosomal DNA, this DNA would then govern the protein via DNA-dependent microsomal RNA. Alternatively, suppose autonomy of this RNA: mutations in it would then be propagated so that cells with a stable DNA gene would include many varieties of RNA. Rather than the whole cell, each of the ribosomes would stand as a unit of function, propagation, and selection.

The elective role of the inducer in the synthesis of β-galactosidase in E. coli has been greatly clarified by the recent work of Pardee, Jacob, and Monod (C. R. Acad. Sci. 246:3125-3128, 1958.) They now propose that the inducer releases a preformed enzyme-forming system from internal repression. The de-repression may depend, in part, on competitive complex-formation by inducers with the incipient enzyme to facilitate its release from its RNA template. The same concept might apply to A7. The introduction of a homologous antigen might stimulate an antibody-forming cell by releasing incipient antibody from its complexes with RNA.

Neolactose is referred to in a misleading way: this compound is a good substrate but a poor inducer. The discrepancies may be eventually cleared up if the incipient enzyme, bound to the template, has somewhat different specificities from the free enzyme.

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