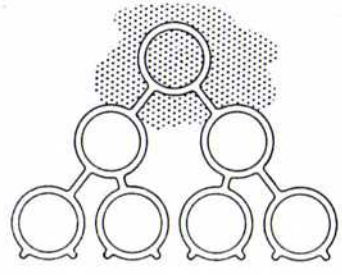
PREVIOUS FLEXNER LECTURES

HEINRICH POLL (Germany) *Melos or The General Structure in Biology*	Fall, 1928
SIR WILLIAM BATE HARDY (England) *To Remind; a Biological Essay*	Spring, 1931
FRANCIS RICHARD FRASER (England) *The Principles of Therapeutics*	Winter, 1933
GUNNAR NYSTROM (Sweden) *Embolism and Other Surgical Subjects*	Spring, 1935
THORVALD MADSEN (Denmark) *The Epidemiology and Control of Syphilis, Tuberculosis, and Whooping Cough and Other Aspects of Infectious Disease*	Spring, 1937
ALBERT V. SZENT-GYORGI (Hungary) *On Oxidation, Fermentation, Vitamins, Health and Disease*	Spring, 1939
DONALD D. VAN SLYKE (U.S.A.) *Physiology of the Kidney and Renal Function in Diseases of the Kidney*	Spring, 1942
WARFIELD LONGCOPE (U.S.A.) *Clinical Nephritis*	Spring, 1942
H. J. CURTIS (U.S.A.) *Excitation and Conduction in Nerve*	Spring, 1945
OLIVER H. LOWRY (U.S.A.) *Microchemical Methods for the Appraisal of Nutritional Studies*	Spring, 1945
ROBERT F. PITTS (U.S.A.) *Renal Regulation of Acid Base Balance with Special Reference to the Nature of the Mechanism for Acidifying the Urine*	Spring, 1945
H. A. BLAIR (U.S.A.) *Pneumocardiography*	Spring, 1945
SIR EDWARD MELLANBY (England) *A Story of Nutritional Research: The Effect of Some Dietary Factors on Bones and the Nervous System*	Spring, 1947
ARVID WALLGREN (Sweden) *Tuberculosis and Other Problems of Paediatrics*	Spring, 1949
HANS LUDWIG KOTTMEIR (Sweden) *Carcinoma of the Female Genitalia*	Fall, 1951
WILLI HOFFER (England) *Psychoanalysis: Practical and Research Aspects*	Fall, 1953
J. HAROLD BURN (England) *Functions of Autonomic Transmitters*	Spring, 1956

THE ABRAHAM FLEXNER LECTURES * 1958

THE CLONAL SELECTION THEORY OF ACQUIRED IMMUNITY



Frank
SIR MACFARLANE BURNET

VANDERBILT UNIVERSITY PRESS * NASHVILLE 1959

PUBLISHED IN GREAT BRITAIN BY
CAMBRIDGE UNIVERSITY PRESS

DIENES, L. (1929). *J. Immunol.* **17**, 531.
DIXON, F. J. & MAURER, P. H. (1955). *J. exp. Med.* **101**, 245.
DIXON, F. J., WEIGLE, W. O. & ROBERTS, J. C. (1957). *J. Immunol.* **78**, 56.
FAGRAEUS, A. (1948). *Antibody production in relation to the development of plasma cells* (Stockholm).
HUMPHREY, J. H. & PORTER, R. R. (1957). *Lancet*, **1**, 196.
JETER, W. S., LAWRENCE, K. A. & SEEBOHM, P. M. (1957). *J. Bact.* **74**, 680.
KABAT, E. A. (1956). *J. Immunol.* **77**, 377.
KUHNS, W. J. & PAPPENHEIMER, A. M., Jr. (1952). *J. exp. Med.* **95**, 363.
LOVELESS, M. H. (1940). *J. Immunol.* **38**, 25.
LOVELESS, M. H. & CANN, J. R. (1955). *J. Immunol.* **74**, 329.
MAY, J. K. & WEISER, R. G. (1956). *J. Immunol.* **77**, 34.
MCMASTER, P. D. & HUDACK, S. S. (1935). *J. exp. Med.* **61**, 783.
RAFFEL, S. & FORNEY, J. E. (1948). *J. exp. Med.* **88**, 485.
ROHN, R. J., BEHNKE, R. H. & BOND, W. H. (1954). *J. Lab. clin. Med.* **44**, 918.
ROITT, I. M., DONIACH, D., CAMPBELL, P. N. & HUDSON, R. V. (1956). *Lancet*, **2**, 820.
SHERMAN, W. B., MENZEL, A. & SEEBOHM, P. M. (1950). *J. exp. Med.* **92**, 191.
TALIAFERRO, W. H. (1949). *Annu. Rev. Microbiol.* **3**, 159.
WESSLEN, T. (1952). *Acta derm.-venereol., Stockh.*, **32**, 195, 245.
WOODRUFF, M. F. A. & WOODRUFF, H. G. (1950). *Phil. Trans.* B, **234**, 559.

IV

THE CLONAL SELECTION THEORY OF ANTIBODY PRODUCTION

In the last chapter I gave a short account of what I regarded as the important experimental and observational facts of the immune responses. In this, I want to discuss the possible ways in which a general theoretical account of the findings can be constructed. It would be difficult, presumptuous, and inappropriate to this series of lectures to attempt to deduce directly from the facts the most likely theory of antibody production. Instead, I shall consider only points of view that have already been expressed, beginning with Ehrlich's side-chain theory.

1. *The side-chain theory of Ehrlich*

This is, of course, based on an old-fashioned picture of cell metabolism, but its essence can be expressed in terms that would be intelligible today. Ehrlich assumed that all foreign antigens, and especially the bacterial toxins with which he was primarily concerned, damaged body cells by combining with pre-existent chemical patterns (side chains) normally concerned with some metabolic function. The union was specific and irreversible and the damage to the cell could only be overcome by casting off the blocked side chain and replacing it with a new one. Such regeneration was regarded as conforming to the general law of over-compensation that had been formulated by Weigert, and would therefore tend to produce an excess of side chains which were liberated into the body fluids.

The side-chain theory implies the existence of cellular patterns complementary to all possible antigenic determinants. With Landsteiner's studies of serological specificity

it became clear that an enormous variety of cell receptors would need to be provided, so many that Ehrlich's theory was discarded. Landsteiner in 1936 concluded that antibodies are produced by some modification of the process of protein synthesis by which the proteins (antibodies) are adapted as closely as possible to the immunizing antigen.

2. *Haurowitz–Pauling (direct template) theory*

With the development of a better understanding of protein chemistry, Landsteiner's work became the basis for the first of the current theories of antibody production, for which Alexander, Mudd and Haurowitz are usually given joint credit. Its most fully developed form is due to Pauling (1940) and it is probable that most immunologists still find it the most convenient framework for thought on practical matters. Following Talmage (1957), the theory can be called the 'direct template' theory. It holds that antibody molecules have their specificity determined by being synthesized against a template of the antigen molecules themselves. In Haurowitz's (1952) view the specificity is conferred not at the synthesis of the polypeptide chain but at a later stage when the folding of the chain is taking place. In this way a complementary configuration is developed at one or more, probably two, sites on the coiled-up globulin, which 'fits' accurately with the antigen or its particular determinant against which it was moulded.

It is doubtful whether anyone has attempted to cover all the main fields of immunology in relation to this view. Clearly accessory hypotheses not very closely dependent on the primary one are needed to deal with normal antibodies, immunological tolerance, homograft immunity, etc. Its chief virtue has been the stimulus it has provided for the experimental study of specificity of chemical structure, and of the persistence of foreign organic material in various tissues. The important implications of this theory in which it differs from others are:

(i) antibody can be produced only while antigen or its determinants remain as such in the tissues:

(ii) antibody can be produced against any type of organic pattern provided it is presented on an appropriate carrier macromolecule, most commonly protein in character.

3. *Burnet–Fenner indirect template theory*

In 1949, Burnet and Fenner published a comprehensive discussion of the available information in terms of what has been referred to as an 'indirect template' theory. This was elaborated but not basically altered by Burnet in 1956.

The theory was an attempt to include in a general formulation those features for which the direct template theory has no point of contact. We held, for instance, that the two most important features of immune reactions were (*a*) that body components are immunologically inert and that an equivalent tolerance to foreign antigens can be demonstrated if they are introduced at an appropriate stage in embryonic life and (*b*) that antibody production can continue long after the effective antigen has disappeared from the body.

The indirect template theory still adopted the view that antibody production was an active response of cells to the entry of foreign organic material, and for this theory the existence of natural antibody was an accidental and irrelevant circumstance. The new features were essentially three in number:

(i) To account for the non-antigenicity of body components these were assumed to carry 'self-markers'; at some point in the antibody-producing sequence a 'recognition unit' was postulated to act as a means of detecting material carrying self-markers and deflecting it from the possibility of immune response;

(ii) To account for the persistence of antibody-producing capacity it was postulated that a 'genocopy' of the antigenic determinant was incorporated in the genome

of the stem cell concerned, so allowing the indefinite production of descendant antibody-producing cells;

(iii) This incorporation of pattern determinants into the genetic structure of antibody-producing cells provided some basis for the changes in antibody character that may result from secondary antigenic stimuli or simple lapse of time.

This theory had its main success in predicting that immunological tolerance following prenatal injection of appropriate antigens should be experimentally demonstrable. On the other hand, the concept of self-markers and recognition units was seen to be a clumsy one that could only be a rough paraphrase of the actual mechanism.

4. *Jerne's natural selection theory*

In 1955 Jerne published a new and strikingly different conception of antibody production in which, for the first time since Ehrlich, natural antibodies were seriously considered in relation to 'true' antibodies. Jerne discarded altogether the view that antibody production was a direct result of the entry of an antigen into body cells. He held that the gamma globulin molecules of the plasma represent a population comprising carriers of all the reactive sites needed to unite with *any* potential antigenic determinant except those already existing in accessible components of the body. The function of the antigen which enters the body from without is to act as 'a selective carrier of spontaneously circulating antibody to a system of cells which can reproduce this antibody'. It is assumed that once antibody is taken into cells of the antibody-producing system, replicas of this natural antibody will be produced. With the liberation of this crop of new antibody a second injection of antigen will find many more antibody-producing cells and give a stronger 'secondary' stimulus to antibody production. Like the direct template theory, the natural selection theory leaves a considerable range of questions unanswered. It is not clear, for instance,

whether the multiplicity of antibody reactivities in gamma globulin molecules is a random character due to a simple indeterminacy of pattern in the molecules produced or whether there are clones of cells which produce pattern *a*, others pattern *b*, and so on for the thousands of patterns required. This second possibility would naturally also demand a corresponding range of coding for pattern in the nucleic acids of the cells forming the clones in question. Jerne appears to favour the first alternative and to assume that when a (partially denatured) globulin molecule which happens to have pattern *x* is brought to any one of the antibody-producing cells this cell is stimulated to produce globulin of *definitive* pattern corresponding precisely to the random pattern of the 'natural' antibody molecule. This appears to be out of line with any of the current ideas on protein synthesis and is one of the main points which led to the development of a 'clonal selection' theory.

5. *The clonal selection theory*

The great contribution of Jerne's theory was that it drew attention to the theoretical possibility that the recognition of self from not-self could be achieved in another fashion than by the recognition of 'self-markers'. As Talmage (1957) points out, Ehrlich's side-chain theory was in many ways the logical equivalent of Jerne's concept. The side-chain theory was quietly shelved as evidence accumulated of the vast variety of antibodies that could be produced, some against non-biological determinants such as arsanilic acid. It seemed, and to most immunologists still seems, inconceivable that all types of antibody could be pre-existent in the normal complement of gamma globulin molecules. Nevertheless, if Jerne is correct that a comprehensive range of molecules corresponding to all organic patterns other than those of body components is present in the gamma globulin population, this would be an effective and much more elegant way of accounting for the differentiation of self from not-self.

The outstanding difficulty in accepting Jerne's theory is the claim that when a given type of natural antibody molecule is brought to a cell by antigen, the cell then proceeds to make more natural antibody molecules of the same type. The facts that, in general, union with specific antigen results in partial denaturation of antibody globulin, that there is no nucleic acid in antibody and that homologous antibody is very rapidly broken down when it is taken into a cell (Humphreys and Macfarlane, 1954), all speak against the concept. Talmage (1957) pointed out that it would be more satisfactory if the replicating elements essential to any such theory were cellular in character *ab initio* rather than extracellular protein which can replicate only when taken into an appropriate cell. He did not elaborate this view but clearly regards it as the best current basis for immunological theory. Our own view is that any tenable form of Jerne's theory must involve the *existence of multiple clones of globulin-producing cells*, each responsible for one genetically determined type of antibody globulin. This immediately poses the question of how the antibody–antigen complex can reach the cells, which are genetically determined to produce the corresponding type of antibody molecule. Clearly it would simplify matters a great deal if the antigen were in a position to react with natural antibody or a pattern equivalent thereto on the surface of the cell which produced it.

This is the crux of the clonal selection hypothesis. It assumes that in the animal there exist clones of mesenchymal cells, each carrying immunologically reactive sites corresponding in appropriate complementary fashion to one (or possibly a small number of) potential antigenic determinants. This provides a population of cells which, when an appropriate stage of development has been reached, are capable of producing the population of globulin molecules which collectively provide the normal antibodies. When an antigen is introduced it will make contact with a cell of the corresponding clone, presumably a lymphocyte, and by so doing

stimulate it to produce in one way or another more globulin molecules of the cell's characteristic type. The obvious way of achieving this is to postulate that stimulation initiates proliferation as soon as the cell in question is taken into an appropriate tissue niche, spleen, lymph node or subacute inflammatory accumulation.

The reasons for temporarily or permanently discarding the 'indirect template' hypothesis in favour of this clonal selection approach were cumulative and largely indirect.

(i) In discussing the biology of malignant disease the importance of clonal phenomena in the expendable cells of the body became increasingly evident (Burnet, 1957*b*).

(ii) Interest in the auto-immune complement fixation test developed in my laboratory by Gajdusek (1957) led to the tentative conclusion that the 'antibody' concerned was an adventitious mixture of globulin molecules produced by clones of cells which had undergone some type of somatic mutation.

(iii) Workers in the field of adaptive enzyme production in bacteria are now unanimous that the pattern of the adaptive enzyme is genetically determined and not a 'transcript' of pattern introduced by substrate or inducer molecule. This destroyed the significance of any analogy between adaptive enzyme production and the indirect template hypothesis of antibody production.

(iv) Changing views on the life-history of the lymphocyte have made it admissible to postulate that a lymphocyte appropriately stimulated could give rise to a clone of descendant cells. In particular, work from Florey's laboratory (Gowens, 1957) shows that lymphocytes can undergo more than one cycle between tissue and circulation. Simonsen (1957), working with avian material, showed that adult fowl blood contained circulating cells which could settle and proliferate in

embryo spleen and produce antibody against the new host.

(v) Several immunologists, including Jerne himself, have suggested that the self-marker theory is semi-mystical in character and generally unattractive.

For these reasons a sketch of what appeared to be necessary modifications in Jerne's theory was prepared (Burnet, 1957*a*) and found to have some interesting potentialities, particularly in relation to the pathology of immune reactions in man. As in every such theoretical elaboration, a number of *ad hoc* assumptions had to be made, but it is felt that all of these are in line with the basic concept. This may be stated from a more generalized viewpoint as follows:

The antibody-producing cells of the body make up a mobile population of mesenchymal cells constantly undergoing physiological and mutational change. It is composed of large numbers of clones from which subclones are constantly arising as a result of somatic mutation.

Individual clones prosper or dwindle in accord with their experience of contact with the corresponding antigenic determinants. The result of such contact will depend on a variety of physiological considerations of which the most important is probably the age of the individual concerned. The fate of these clones can legitimately be regarded as a study in the population genetics of mesenchymal cells in the internal environment of the whole organism.

(*a*) *Developmental considerations*

The development of the starting population of mesenchymal cells present at birth or hatching is part of the general problem of differentiation and requires a number of additional *ad hoc* assumptions which, however, are of the same general quality as are needed for the provisional interpretation of any phase of embryonic differentiation.

Perhaps the clearest way to present the processes that must be postulated during embryonic development is to consider

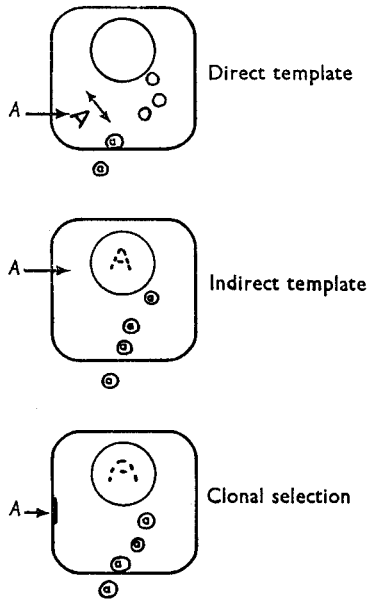


Fig. 6. A diagram to suggest the essential differences between the three theories.

In the first (direct template theory) the antigen itself enters the cell and stamps a complementary pattern on each globulin molecule after it has been produced.

In the indirect template theory the antigen, as it were, incorporates an image of itself in the genetic mechanism of the cell. This is suggested by putting a broken *A* in the nucleus—but the possibility of a cytoplasmic mechanism of inheritance might appeal more to others.

In the clonal selection theory, the image of the antigenic determinant is present, shadowy perhaps, in the genetic mechanism before first contact with antigen, which can stimulate, but not effectively enter, the cell. The image and the globulin pattern can be sharpened up or modified by mutation and selection.

the most conspicuous of the normal antibodies, the isoagglutinins anti-*A*, anti-*B* and anti-*H*(*O*).

There are still conflicting opinions in regard to the significance of the *iso*agglutinins but, having regard to the phenomena of immunological tolerance, that suggested by Burnet and Fenner (1949) is still the most probable. They

suggested that there were normal globulin-producing cells which 'by accident' were potentially capable of producing molecules with the configurations needed to act as *iso*-agglutinins of the two types (and we should now add anti-*H* as well). If, however, the genetic constitution of the embryo resulted in the production of *A* substance on red cell surface or elsewhere, the potential producers of anti-*A* were rendered tolerant; that is, *A* was recognized as self and only anti-*B* developed. The other groups would be similarly interpreted. Two haematological anomalies provide rather strong support for this view. The human chimaera described by Dunsford *et al.* (1953) was genetically *O* with *A* cells from her twin brother derived presumably by prenatal implantation of haematopoietic cells. She showed no anti-*A* *iso*agglutinin and the *A* cells were negative to Coombs' test. It seems that the capacity to produce anti-*A* which was genetically present must have been inhibited or annulled during embryonic life. The converse situation is to be found in persons with the rare 'Bombay' blood group anomaly (Bhende *et al.* 1952). In this phenotype the cells are not agglutinated by any *iso*agglutinin, presumably as a result of genetic anomaly. The serum contains anti-*A*, anti-*B* and anti-*H*.

This can be taken as a prototype of the kind of interpretation to be adopted in developing the present modification of Jerne's theory. How clones to cover all possible antigenic determinants can arise must be left for later discussion. We assume simply that this does take place at some stage in embryonic life and that mesenchymal cells, presumably lymphocytes, begin to circulate with the characteristic pattern on their surface but do not liberate antibody-type globulin. In the Burnet–Fenner theory, any potentialities of antibody production against body components were eliminated by the development of tolerance. In the present theory they are more readily disposed of by assuming that—at this particular stage of embryonic life—contact with the corresponding antigen pattern results in the death of the cell.

If the potential antigen persists long enough in high enough concentration, all clones which can produce this natural antibody will be eliminated. This provides a simple explanation for the red cell *iso*agglutinins and could obviously be extended to cover all examples of prenatal tolerance. Self–not-self recognition means simply that all those clones which would recognize (that is, produce antibody against) a self component have been eliminated in embryonic life. All the rest are retained.

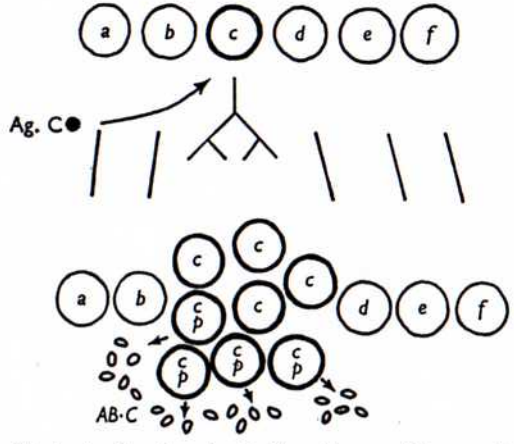


Fig. 7. To illustrate the clonal selection theory of immunity. Contact of the corresponding antigenic determinant *Ag.C* with cells of clone *c* stimulates proliferation to antibody-producing plasma cells *cp* and non-antibody producing types *c*.

To obtain such a result a fairly complex developmental sequence must be postulated. The first point to be considered is how a complete sequence of globulin patterns capable of reacting with all possible determinants could be established. There is a possible clue to be found in the fact that all substances capable of acting as antigens are susceptible to enzymic breakdown. Enzymes are proteins and it is probably a reasonable deduction that proteins have the potentiality of carrying steric patterns complementary to every chemical

configuration that has been produced by living organisms. It may be suggested as a corollary to this that if at a certain stage of embryonic development certain synthetic elements in mesenchymal cells were 'randomized', the possibility might well emerge of producing all the 10,000 or more patterns required.

Table 2. *A representation of the process of immunological maturation*

Embryonic	Phase of differentiation or randomization
	Stabilization of patterns
	Development of tolerance by elimination of reacting clones
Birth or hatching →	←Critical point of Medawar
	Maturation
Post-natal	Liberation of natural antibodies and potentiality of antibody production
	↓ Development

Suppose that at the appropriate stage of development a limited genetic determinant carrying the coding responsible for globulin pattern releases control in such a fashion that purely random arrangements are allowed which will be different at each replication. Then at a later stage control is re-established and the various random sequences are stabilized as the guides to the genetic control of globulin synthesis by the cell line concerned. This is still during embryonic life when gamma globulins are not liberated into the blood and are produced only to the extent necessary to bring their specific patterns to the surface of the cell. It is at this stage that contact with any determinant associated with

a body component or any foreign determinant artificially introduced results in the elimination of cells carrying such sites, and if all such clones are eliminated full tolerance is established.

The next phase is the gradual change in the response to contact with antigenic determinant. From the lethal effect seen in the early stage it changes, perhaps with an intermediate phase of damage and partial recovery, to stimulation of protein synthesis and, in the appropriate tissue environments, active proliferation to produce plasmacytoid cells and lymphocytes with active antibody-liberating capacity.

This sequence, shown schematically in Table 2, provides the outline of what can be called the clonal modification of Jerne's hypothesis or the clonal selection theory.

6. *Comparison of theories*

In comparing the four modern theories that I have outlined, the first requirement is a decision over the fundamental difference between the direct template theory and the other three. If antigenic determinants are needed to take a direct part in all antibody production, then there is no scope for discussion of the problem along genetic lines and no way of providing an interpretation of such phenomena as the non-antigenicity of self-components, immunological tolerance, cell-borne antibody or modification of antibody character with time and differing degrees of stimulation. As a *general* hypothesis to cover immunological behaviour, this theory is manifestly inadmissible. The only question that really arises is whether, on a background of one of the other three hypotheses, a new mechanism has been superimposed by which production of urgently needed protective substance may be accelerated. This might take the form of an enforcement of somatic mutation in the required direction in those cells whose natural pattern fitted best for this function. This would be a rather unhappy hybrid.

For reasons that have already been given, we believe that

Jerne's theory in its original form is inadmissible until it has been provided with a cellular basis. It then becomes identical with the clonal selection hypothesis (theory 5). The common feature of the two 'biological' approaches, the indirect template hypothesis (3) and the clonal selection hypothesis (5), is that once antibody-producing capacity is implanted in a clone, cells of that clone respond by activation and proliferation when they have new contact with the appropriate antigen. This provides an evolutionary situation within which clones will wax and wane in size and activity in ways analogous to a mixture of bacterial clones in a common but changing environment.

A direct comparison between the indirect template theory and the clonal selection theory is more difficult than at first sight would seem likely. Once a potential antibody-producing cell has been modified by the antigen so that it can give rise to antibody-producing descendants, we have a clonal situation even with the indirect template theory. The only essential difference between theories (3) and (5) is simply the origin of the primary specific patterns. In the clonal selection theory (5) they are pre-existent as a result of processes of embryonic development. The indirect template theory (3) gives each macrophage an inbuilt and somatically inheritable recognition mechanism which allows it to recognize and inactivate body components, perhaps by breaking them down into inert small molecular fragments. Unrecognized (foreign) macromolecules pass to the nucleus and induce genetic modification. The modified character is then passed to a cell of the lymphoid series, either by some mechanism resembling transduction or by actual conversion of the modified cell to a lymphoid stem cell.

Perhaps the best way to summarize the position is to say that there are two sets of observable phenomena which are crucial to the understanding of antibody production.

(i) The non-antigenicity of body components and the phenomena of prenatally induced tolerance.

(ii) The persistence of immunological 'memory' over many years—for example, Davenport's phenomenon (Davenport and Hennessey, 1956).

In addition, there is (iii), an accepted principle derived largely from modern work on adaptive enzyme formation in bacteria that the 'code' determining the pattern of all functional proteins is genetically provided.

The last of these is perhaps not to be regarded as a categorical requirement until a great deal more is known about genetic aspects of differentiation, but it would seem most unwise to disregard the principle unless absolutely forced to do so.

If we adopt it, we can in the first place accept some of the differences between individuals in the type or amount of antibody produced as being related to inborn genetic differences. Qualitative differences amongst gamma globulin molecules produced by the same individual may be ascribed (*a*) to the process of embryonic differentiation, (*b*) to somatic mutation at any time during life. The equally or more important differences in the relative numbers of different types of gamma globulin molecule can be ascribed to (*a*) selective proliferation of cells of certain clones as a result of specific stimulation, (*b*) activation to produce and liberate globulin of cells of appropriate clones, (*c*) transfer of genetic character from cells of one clone to those of another by some process of transduction in the broad sense.

Any hypothesis to incorporate such a point of view must take the form of a clonal selection theory. That can hardly be a matter for controversy. The principal difference of opinion will be whether it is or may become necessary to accept more active participation of foreign antigens in determining the emergence of antibody-producing clones—in other words, to graft something of the indirect template theory on to the clonal selection hypothesis. The possibility cannot be eliminated and if new experiments make it necessary to adopt such a point of view, the remoulding of genetic

mechanism might be visualized in the fashion discussed by Burnet (1956) and by Schweet and Owen (1957). At the present time, however, there seems no reason to engraft such a grossly Lamarckian qualification on what might be described as a strictly Darwinian process at the cellular level.

7. *Cellular reactivity required by a clonal selection theory*

The mechanism by which contact with antigen can influence a cell of the corresponding clone must probably be sought from analogies with the various results that have been described by which antigen–antibody reaction will damage cells. The classic examples are immune haemolysis and the anaphylactic response. Haemolysis requires complement and in the classical demonstration of anaphylaxis by the Schultz–Dale procedure no addition of complement is necessary, though Becker (1957) feels that the presence of adsorbed complement on the reacting cells cannot be excluded. None of the reactions is yet adequately understood but it appears to be a valid generalization that reaction of antibody with antigen either incorporated in or attached to the cell surface initiates a process leading to some breakdown in the integrity of the cell surface with leakage of cell contents. In general the character of the response will depend on the type of cell; red cells can show virtually only haemolysis, mast cells liberate heparin, serotonin and histamine, while it seems likely that other mesenchymal cells may suffer damage of various degree up to necrosis. It seems likely that parenchymal cells are not affected directly by antigen–antibody reaction but only by the pharmacologically active agents liberated by the more generalized mesenchymal cells.

It is known that immunological reactivity can vary widely according to such factors as the age of the subject, the degree of exposure to antigen and the level of circulating steroid hormones. There is therefore no reason against the postulate that the type of reactivity changes characteristically with maturation of the body in the immunological sense. All that

is being assumed is that each immunologically conditioned cell carries at some accessible sites reactive groupings equivalent to those by which an antibody globulin molecule attaches to the specifically corresponding antigenic determinant. When a reactive site makes specific adsorptive contact with an antigenic determinant, this union is assumed to act as a trigger initiating intracellular processes whose character and result will depend on the various factors already mentioned.

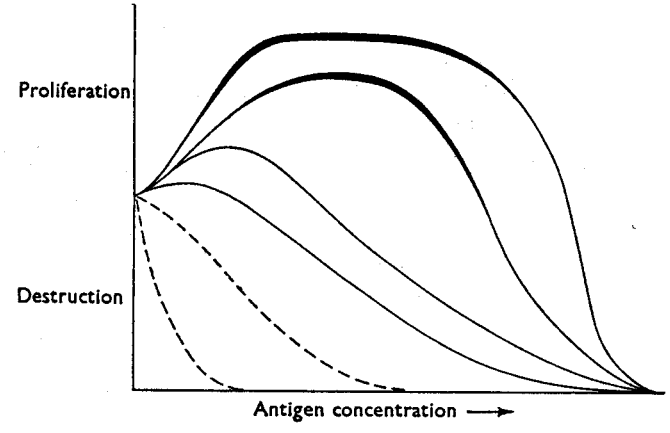


Fig. 8. The changing reactivity postulated for immunologically conditioned cells at various stages of maturity. (See text.)

Figure 8 has been devised to indicate the types of reactivity which need to be postulated to cover the various phenomena of immunological tolerance and of antibody production. The ordinates correspond to the main types of reactivity that are significant from the point of view of cell numbers. The zero point corresponds to no reaction, above this proliferation, and—where the lines are thickened—the development of plasma cells and antibody production, are indicated. Below the zero point we have inhibition of proliferation extending down to complete dormancy or destruction. The abscissae represent effective concentration of antigenic determinants. This will almost certainly be a complex function in which

'goodness of fit' between reactive site and antigenic determinant plus the accessibility of the receptors to antigenic determinants under the existing conditions will play a part as well as usual measures of concentration of antigen.

The family of curves, beginning with the most steeply sloping broken line, is intended to represent immunological reactivity of immunologically conditioned cells at various stages of the process of maturation. It is assumed without proof that curves shown in broken lines would depict eventual destruction of a stimulated cell, while continuous lines represent persistence of the cell line.

This diagram is of special interest in relation to work on the induction of non-reactivity by administration of a single dose of a pure antigen to rabbits at birth (Smith, 1958). Under these circumstances so long as an appropriate number of antigenic determinants are in contact with a cell, the cell is rendered dormant in two senses: (*a*) it is incapable of developing into a plasma cell and producing antibody and (*b*) it remains at the same stage of immaturity as it was at the time of first contact with antigen.

In any workable form of the clonal selection theory it will be necessary to consider reaction with related patterns. All that is demanded of the primary differentiation or randomization of pattern is that a sufficient number of clones are produced to provide sites with a *close enough* fit to all possible antigenic determinants to allow some reaction. As long as it is possible by directed protein synthesis to secure a better fit, it is legitimate to look to minor somatic mutation to allow its emergence.

If, in fact, the concept of randomization of pattern in embryonic life is accepted, or even a complex genetically determined output of multiple globulin patterns arising by differentiation, then it would be in accord with biological probabilities to find an occurrence of relatively frequent (somatic) mutation in the range of loci concerned in this particular function of specifying pattern of gamma globulin.

The combination of frequent minor mutation and a highly effective selective process would rapidly improve the accuracy of the complementary relationship to new antigenic determinants. The final distribution of patterned globulin molecules in the adult animal would on this view come to be almost wholly determined by the immunological history of the individual. Such a view would provide opportunity for many functional variations depending (*a*) on the completeness or otherwise of the initial complement of prototype clones, something which might well be determined by the genetic make-up of the individual; (*b*) on changing capacity to mutate with advancing age. It may be relevant that Sabin *et al.* (1947) found that elderly Japanese men produced an antibody against Japanese encephalitis *B* vaccine only if they had evidence of past infection by the virus.

8. *Summary*

The clonal selection theory of antibody production is based on the concept that the antibody-producing cells of the body form part of a mobile population of mesenchymal cells constantly undergoing physiological and mutational change.

When mutational change occurs a new clone is initiated. It is postulated that mesenchymal cells carry surface sites analogous to the specific patterns on the antibody globulins they produce. Stimulation by contact of these sites with the corresponding antigenic determinant may provoke more than one type of response, but the crucial one is the proliferative response which allows a selective advantage to the clone concerned.

The theory differs from the standard interpretation of antibody production in replacing the concept that in one way or another the antigen actively enforces production of a new pattern of specific globulin, by the view that somatic mutation and selection within the mesenchymal cell population can have the same overall effect.

REFERENCES

BECKER, E. L. (1957). *J. cell. comp. Physiol.* **50**, Suppl. 1, 303.

BHENDE, Y. M., DESHPANDE, C. K., BHATIA, H. M., SANGER, RUTH, RACE, R. R., MORGAN, W. T. J. & WATKINS, WINIFRED M. (1952). *Lancet*, **1**, 903.

BURNET, F. M. (1956). *Enzyme, Antigen and Virus* (Cambridge).

BURNET, F. M. (1957*a*). *Austral. J. Sci.* **20**, 67.

BURNET, F. M. (1957*b*). *Brit. med. J.* **1**, 779, 841.

BURNET, F. M. & FENNER, F. (1949). *Production of Antibodies* (2nd ed. Macmillan, Melbourne).

DAVENPORT, F. M. & HENNESSEY, A. V. (1956). *J. exp. Med.* **104**, 95.

DUNSFORD, I., BOWLEY, C. C., HUTCHINSON, A. M., THOMPSON, J. S., SANGER, R. & RACE, R. R. (1953). *Brit med. J.* **2**, 81.

GAJDUSEK, D. C. (1958). *Arch. int. Med.* **101**, 9.

GOWENS, J. L. (1957). *Brit. J. exp. Path.* **38**, 67.

HAUROWITZ, F. (1952). *Biol. Rev.* **27**, 247.

HUMPHREYS, J. H. & MACFARLANE, A. S. (1954). *Biochem. J.* **57**, 186, 195.

JERNE, N. K. (1955). *Proc. nat. Acad. Sci., Wash.*, **41**, 849.

LANDSTEINER, K. (1936). *Specificity of Serological Reactions* (Baltimore).

PAULING, L. (1940). *J. Amer. chem. Soc.* **62**, 2643.

SABIN, A. B., GINDER, D. R., MATUMOTO, M. & SCHLESINGER, R. W. (1947). *Proc. Soc. exp. Path.* **65**, 135.

SCHWEET, R. S. & OWEN, R. D. (1957). *J. cell. comp. Physiol.* **50**, Suppl. 1, 199.

SIMONSEN, M. (1957). *Acta path. microbiol. scand.* **11**, 480.

SMITH, R. T. (1958). *Fed. Proc.* **17**, 535.

TALMAGE, D. W. (1957). *Annu. Rev. Med.* **8**, 239.

V

IMPLICATIONS OF THE CLONAL SELECTION HYPOTHESIS

The preceding chapter contains a general account of the clonal selection hypothesis. In the next three chapters an attempt is made to examine in some detail a rather wide range of immunological topics in terms of the hypothesis.

The primary objective was, of course, to find situations from which it could be deduced that one or other of the current hypotheses was clearly inadmissible. So far this has proved impossible of accomplishment.

All experimental work on antibody production has necessarily been compelled to employ large natural populations of cells of diverse origins irrespective of whether experiments are carried out *in vivo* or *in vitro*. Under these circumstances it seems unlikely that any experimental decision will be possible as between the indirect template hypothesis and the clonal selection hypothesis. It is easily seen that once the capacity to produce antibody has been developed in a cell the implications of the two theories are virtually identical. The difference concerns only the way in which a primary immunization is effected. Is the new pattern produced by a direct impact of antigenic determinant pattern on the protein synthetic mechanism—or does the antigenic pattern act purely as a selective agent on material provided by spontaneous mutational processes? Only by the use of a pure clone technique of tissue culture which allowed mesenchymal cells to retain full functional activity would we be likely to find an answer.

The clonal selection hypothesis would be completely validated if it could be shown that single cells from a non-immune animal gave rise to clones, each cell of which under proper physiological conditions contained, or could liberate,