70th Anniversary of Folia Biologica

TOMÁŠ ZIMA, JAN ŽIVNÝ, ZDENĚK KLEIBL

Folia Biologica celebrates 70 years of continuous publication of research papers. The first volume was published in Prague in 1954 on behalf of the Institute of Molecular Genetics of the Czechoslovak Academy of Sciences (since 1990 the Academy of Sciences of the Czech Republic) under the subtitle "International edition of the journal Czechoslovakian Biology". Born in the dark days of the Cold War, Folia Biologica provided a thin but important link between the politically controlled science behind the Iron Curtain in the former Czechoslovakia and that of the free Western world. Initially, the journal focused on research papers in the fields of experimental medicine, immunology, virology, and experimental zoology. Since 1961 (Volume 7), Folia Biologica has been indexed in the Web of Science database. The first issue of Volume 7 was introduced by a review article by Peter Brian Medawar (1915–1987), winner of the 1960 Nobel Prize in Physiology or Medicine "for the discovery of acquired immunological tolerance", which is reprinted in this anniversary issue [1].

In the late 1960s, during the political relaxation that culminated in the Prague Spring, cooperation with free Western science intensified and enabled a lively scientific dialogue between Czechoslovak and foreign biological scientists, namely immunologists, molecular biologists, and virologists, as illustrated by a series of original research articles from Folia Biologica by Georg Davis Snell (1903–1996) and Jean Dausset (1916–2009) (Table 1), who were awarded the Nobel Prize in Physiology and Medicine in 1980 "for their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions", which led to the discovery of the major histocompatibility system (MHC) [2–7]. Another powerful example is an article in Folia Biologica by François Jacob (1920–2013), who was awarded the Nobel Prize in 1965 for discoveries that helped elucidate the transcriptional control of enzyme levels [8].

Despite the years of political repression during the "normalization" period following the invasion of the Warsaw Pact troops into Czechoslovakia in 1968, the scientists and editors of Folia Biologica from the Academy of Sciences were able to maintain vibrant contacts with the world's leading scientists. In 1981, the journal changed its subtitle to "Journal of Cellular and Molecular Biology". In 1983, Folia Biologica published the article by Renato Dulbecco (1914–2012), who was awarded the Nobel Prize in 1975 for "discoveries concerning the interaction between tumor viruses and the genetic material of the cell"[9].

With further orientation towards human molecular medicine, the journal entered the era after the Velvet Revolution in 1989, which represented the desired end of political control over national science. The interest of Czechoslovak and Czech scientists in publishing in Folia Biologica began to decline at the end of the 1990s, when they had at their disposal the full range of scientific journals from all over the world. Since volume 63

Table 1. The three most cited original and review articles published in Folia Biologica (from 1961 to 2024).

Article	Year	Times cited
Original research articles		
Pavlícek A, Hrdá S, Flegr J. Free-Tree--freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biol (Praha). 1999;45(3):97-99.	1999	424
Snell GD. The H-2 locus of the mouse: observations and speculations concerning its comparative genetics and its polymorphism. Folia Biol (Praha). 1968;14(5):335-358.	1968	265
Svoboda J, Chyle P, Simkovic D, Hilgert I. Demonstration of the absence of infectious Rous virus in rat tumour XC, whose structurally intact cells produce Rous sarcoma when transferred to chicks. Folia Biol (Praha). 1963;9:77-81.	1963	138
Reviews		
Kostrouchová M, Kostrouch Z, Kostrouchová M. Valproic acid, a molecular lead to multiple regulatory pathways. Folia Biol (Praha). 2007;53(2):37-49.	2007	110
Kodydková J, Vávrová L, Kocík M, Žák A. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. Folia Biol (Praha). 2014;60(4):153-167.	2014	106
Druga R. Neocortical inhibitory system. Folia Biol (Praha). 2009;55(6):201-217.	2009	95

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With the new decade that begins with this issue, the journal has undergone a series of improvements, including the strengthening of the editorial board, the assignment of a DOI (Digital Object Identifier) number to each article, the improvement of the cover layout and graphics, the innovation of the website, and a more precise definition of the journal's aim. Folia Biologica now publishes articles describing original research aimed at elucidating a wide range of issues in biomedicine, especially in oncology and human molecular genetics. In addition, the journal focuses on the cellular and molecular mechanisms of disease and provides studies on all organisms, cells and tissues that serve as biological and disease models, as well as clinical and translational research studies. Further improvements towards sustainable and rapid publication will be accomplished by introducing an online-only publication model planned for 2025.

To celebrate the 70th anniversary of Folia Biologica, we begin the anniversary volume with the reprint of Sir Peter Brian Medawar's review. To commemorate the continuing history of the journal, and to thank our predecessors and contributors, we present the title pages, table of contents, and editorial boards of Folia Biologica by decade, illustrating the changes in research focus, human knowledge, and the evolution of the journal.

We would like to thank all authors, reviewers, editorial board members, editors and managing editors involved in the journal production in the past decades, namely Ivan Málek, Milan Hašek, Alena Langerová, Josef Říman, Jan Bubeník, Jan Svoboda, Emanual Nečas, Karel Smetana Jr. and Zdeněk Kostrouch, for their commitment and dedication to Folia Biologica.

We wish our journal many more decades of scientifically interesting articles, publishing open-minded science by excellent authors for the pleasure of satisfied readers!

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The First Pages
&
Editors by Decades

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FOLIA BIOLOGICA (PRAHA)

Folia biologica, which is a journal of the Czechoslovak Academy of Sciences, publishes papers related to experimental zoology and medicine and in particular to the study of developmental mechanisms in ontogenesis, which are usually considered as belonging to the sphere of cytology, embryology, genetics, immunology, radiobiology and oncology. Folia biologica thus attempts to unite subject matter which, in given developmental stages of biology, is usually divided up among different specialized branches of biology. These all form, however, part of basic general biological problems, including problems of genetic determination, differentiation and morphogenesis, both from the aspect of mechanisms at molecular and cellular level and from the aspect of the functions of the organism as a whole, such as problems of regeneration, transplantation and reproduction

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VOLUME 60

Nobel Prize Winner Talks About Immunology

FOLIA BIOLOGICA

7 : 1—10, 1961.

Theories of Immunological Tolerance *)

P. B. MEDAWAR

The subject of this lecture is one which is very familiar, I think, to almost all of you. I propose to talk about theories of immunological tolerance—not merely of immunological tolerance as we originally used that term in London and here in Prague, but of immunological non-reactivity or immunological unresponsiveness in general. My lecture will be in two parts. I propose first to consider the empirical classification of the various ostensibly different forms of immunological non-reactivity and then, afterwards, to discuss modern theories of the nature of immunity and tolerance in terms of cellular biology, and particularly the more recent speculations of, for example, Burnet and Lederberg.

I.

First, then, the classification of immunologically unresponsive states. I should like to consider five kinds of specific immunological non-reactivity (tab. 1).

Table 1

Specific unresponsive states

1. IMMUNOLOGICAL TOLERANCE produced by a chronic exposure to antigen beginning very early in life.
2. RADIATION-INDUCED TOLERANCE, e.g. semi-permanent acceptance by A-line mice of CBA marrow or skin after exposure to $\sim$ 900 r whole-body irradiation.
3. SULZBERGER-CHASE PHENOMENON. Insensitivity to (e.g.) picryl chloride or dinitrochlorobenzene produced by prior oral administration.
4. IMMUNOLOGICAL PARALYSIS (Felton). Unresponsiveness to pneumococcal polysaccharides produced by high dosage.
5. PROTEIN OVERLOADING PARALYSIS (Dixon) produced by the injection of high doses of (e.g.) bovine serum albumin into adult rabbits.

The first kind of immunological non-reactivity referred to in table 1 is immunological tolerance—a state of non-reactivity produced by exposing the embryo or the very young animal to an antigenic stimulus which is maintained for as long as the state of unresponsiveness is intended to last. I emphasize that tolerance is a *general* immunological phenomenon. It applies not only to the cellular type of immunity, such as we see in the skin homograft reaction, but also, as Dr. Hašek was the first to show, to orthodox humoral immunity, i.e. to the formation of soluble antibodies such as haemagglutinins. Immunological tolerance can be excited by complex cellular antigens, by protein antigens such as bovine serum albumin and also, as we know from the study of human blood group chimeras, by antigens which the work of Morgan and Kabat has shown beyond

*) A lecture given at the Czechoslovak Academy of Sciences, Prague, September 23, 1960 by P. B. Medawar, F.R.S., Jodrell Professor of Zoology, University College, London,

question to be amino-acid polysaccharide complexes. And tolerance applies not merely to the actively acquired immunities, but also, as work in your own laboratories has clearly shown, to the so-called natural immunities.

The second kind of non-reactivity I want to mention is radiation-induced tolerance—the kind of tolerance which is produced when an A-line mouse, for example, is given some 900 roentgens of whole body x-irradiation and is then grafted with bone marrow and perhaps with skin from a mouse belonging to some different strain. Mice treated in this way can sometimes become stable chimeras; they accept homografts from the donors of the bone marrow, and may even accept heterografts. During the past five years tolerance of this kind has been studied all over the world.

The third kind of non-reactivity is less familiar. The phenomenon referred to in table 1 as the Sulzberger-Chase phenomenon is an inhibition of the delayed type of cutaneous hypersensitivity to pure chemical compounds like dinitrochlorobenzene or picryl chloride. Dr. Chase showed that if a guinea pig is fed by mouth, with, for example, picryl chloride, it does not then become sensitized by the application of picryl chloride to the skin.

The fourth is immunological paralysis in the original sense of Felton—the highly specific non-reactivity produced by the injection into mice of relatively large doses of type-specific pneumococcal polysaccharides. Where doses of about one microgram will produce immunity and confer protection against living organisms, doses of 500 micrograms produce no detectable antibodies and confer no protection.

And finally the phenomenon described by the American immunologist Dixon—the specific unresponsiveness produced in adult rabbits by the injection of high doses of protein antigen. For example, if high doses of bovine serum albumin are injected into a rabbit, there is no evidence of a response, whereas small doses evoke orthodox precipitin formation.

These are five apparently different kinds of specific immunological non-reactivity. What kind of evidence is necessary if we are to classify these states of non-reactivity and find out the relationship between them ?.

Table 2

Information needed for a classification of the unresponsive states

1. Is the disappearance of antigen from the blood stream of the rapid (immune) or slow (non-immune) type ?
2. Is the disappearance of passively-introduced specific antibody of the rapid type (indicating the persistence of antigen in a combining form) or of the normal slow type ?
3. Can the unresponsive animal be restored to normal reactivity by the injection of (a) normal lymphoid cells and/or (b) sensitive or immune lymphoid cells ?
4. Do lymphoid cells from unresponsive animals start or resume the formation of antibodies (etc.) after transplantation into a normal environment, or do they remain inert ?
5. Can animals which are already sensitive or immune be rendered unresponsive ?
6. Having regard to the answers to the above questions is it likely or certain that unresponsive animals are making antibodies (or indulging in some comparable immune response) ?

I have put on this slide (tab. 2) a summary of the empirical evidence we need to have if we are to attempt some classification of the immunologically unresponsive states. The first question we want to ask is, I think, this: if one injects an unresponsive or non-reactive animal with specific antigen, is the rate of disappearance of antigen from the blood stream of the rapid type, showing that

some kind of immune response is in progress; or does antigen disappear slowly, exponentially, as would be the case in a normal, non-immunized animal? That then is the first question: how does the unresponsive animal respond to antigen? Does it behave as if it were immune or as if it had not been immunized at all?

The second question refers to the behaviour of specific antibody. Suppose we inject specific antibody into an immunologically non-reactive animal. Does the antibody disappear quickly, so indicating that antigen is still present in a combining form, or does the antibody disappear slowly, at the rate it would in a normal animal?

The third question is: is it possible to restore a non-reactive animal to a state of normal reactivity by the injection into it of lymphoid cells—either of normal lymphoid cells or, as in the procedure known as adoptive immunization, of lymphoid cells which have already been immunized or sensitized against the antigen which the animal tolerates? This is a most important question, and one of the first my colleagues and I attempted to answer in our analysis of immunological tolerance. In practice it is sometimes difficult to answer, because, as you know, the donor of the transferred lymphoid cells must be isogenic with the animal into which the cells are injected.

The fourth question is the converse of the third. Supposing one removes lymphoid tissue—spleen or lymph nodes—from a non-reactive animal and transplants it to a normal animal, will the lymphoid tissue after transplantation to a normal environment start making an immune response, or perhaps give a secondary response after challenge by specific antigen? The answer to this question should give a clear idea of whether the state of non-reactivity was due to a central or peripheral failure of the immune response. Here, too, the donors and recipients of the transferred cells should be isogenic.

The fifth question is: supposing one takes an animal which is *already* immune or already sensitive, is it then possible to make it non-reactive or unresponsive by any of the procedures earlier described?

Finally, when we have studied the evidence available under all these headings, we must try to answer the really important question I have put last: is the apparently unresponsive animal making a response which is thwarted or suppressed or diverted or inhibited; or is it simply not responding at all? If the tolerant or paralysed animal is making no immunological response whatsoever—if the inhibition of response is central and not peripheral—then it can be said to be in a state of *essential non-reactivity.* Alternatively, it may be making a response, the manifestation of which is somehow or other suppressed.

I should now like to show you a slide (tab. 3) tabulating the answers, so far as they are known—and many of the answers are tentative and hesitant—to the six questions I have asked of the five different types of immunological non-reactivity*.

The first point is that it seems impossible to draw any exact distinction between the final states of non-reactivity described as tolerance, radiation tolerance and the inhibition phenomena of Sulzberger and Chase. In all three, so far as the evidence is known, we are dealing with states of *essential* non-reactivity: there is a central failure of the immunological response. Not all the questions can be answered. For example, in animals which have been made tolerant of homografts by the foetal injection of cells, or by bone marrow grafts following whole body irradiation, it is hardly possible to

*) This table is reproduced from my article on *Theories of Immunological Tolerance* in the Ciba Foundation Symposium on Cellular Aspects of Immunity, (pp. 134—139), London 1960; and this article contains the appropriate references. Additional evidence is discussed later in this lecture.

Table 3

	Tolerance	Radiation Tolerance	Sulzberger-Chase	Paralysis (Felton)	Protein Overloading
Rate of decay of antigen	non-immune	non-immune	—	—	non-immune
Rate of decay of passive antibody	normal	normal	normal	rapid	rapid
Is normal reactivity restored by (a) normal (b) immune lymphoid cells ?	(a) Yes (b) Yes	(a) Yes (b) Yes	(a) ? (b) Yes	probably not ?	probably not ?
Behaviour of lymphoid cells from unresponsive animals when transplanted	inert	?	inert	?	?
Can an animal already sensitive or immune be made unresponsive ?	(No)	No	No	Yes	Yes ?
Is the 'unresponsive' animal making an immune response ?	No	No	No	Yes ?	Yes ?

See text for amplifications of and comments on this table.

test the reactivity of lymphoid cells when transplanted into a normal environment—for such animals are chimeras, and their lymph nodes already contain cells of donor origin. In describing the behaviour of lymphoid cells transferred from tolerant animals as "inert", I am in fact drawing on Dr. Smith's experience with rabbits tolerant of soluble protein antigens. With these reservations in mind, we can say that there is no evidence of any essential difference, in terms of cellular reactivity, between the first three forms of unresponsiveness. The last two—paralysis in Felton's sense, and protein overloading paralysis—are much more difficult to classify, because the evidence is incomplete. In both we are dealing with unresponsiveness produced in adult animals by high doses of antigen. At one time most of us believed that such animals did in fact form antibodies, but that these antibodies were bound by the excess of antigen as fast as they were formed, so that although there was an immunological response at the cellular level, there was no net response in the animal as a whole. The first evidence to cast doubt on the interpretation was the important discovery by Dr. Dixon's group that the rate of decay of protein antigen in rabbits which had been overloaded by high doses was of the slow or non-immune type. It is true, as table 3 indicates, that the rate of disappearance of passively introduced antibody is of the rapid type, at least to begin with—but this is not critical evidence, because the situation may obtain only when antigen is present in excess of the quantities needed to induce and maintain paralysis.

Nevertheless, when I prepared table 3 about a year ago (I thought it would be interesting to show it to you in its original form) I still thought it likely that paralysed or overloaded animals were making an immune response. Since then, new evidence has come forward which argues against this view. You will see question marks against the answer to the question relating to the behaviour of 'paralysed' lymphoid tissue transplanted into a normal environment. Dr. Lewis Thomas of the New York University College of Medicine has since told me that work in his department has in fact failed to give any

evidence that 'paralysed' lymphoid tissue starts or resumes antibody formation in a normal environment. Furthermore, Dr. Coons and Dr. Sercarz in Boston have made direct attempts by the fluorescent antibody technique to see if lymphoid cells in paralysed animals are in fact reacting—and they find no evidence that they are doing so (see Sercarz E., Coons A. H.: Nature, *184* : 1080, 1959). I think therefore that, on present evidence, we must concede that paralysed or overloaded animals are *not* making an immune response—in short, that the entries 'Yes ?' and 'Yes ?' in the bottom right hand corner of table 3 should be replaced by 'No ?' and 'No ?'. Certainly we should not now be justified in saying that, at the cellular level, there was any clear distinction between the five types of immunological responsiveness under consideration; and such is Dr. Merrill Chase's conclusion in his masterly recent review of the problem (Ann. Rev. Microbiol., *13* : 349, 1959).

This does not, of course mean that tolerance and paralysis are indistinguishable phenomena—or phenomena distinguishable only by the methods which have been used to bring the state of unresponsiveness about. It might be, for example, that embryonic or neonatal cells—future antibody-forming cells—are specially easy to paralyse; or, if you prefer to put it the other way round, that adult cells are specially difficult to make tolerant. At first sight it seems easy in principle to decide whether or not tolerance and paralysis are in fact distinct: starting with animals of different ages, one would have to find out what dose of antigen, in mg. per kg. per day, was necessary to institute a state of unresponsiveness. Experiments of this kind are in progress in Dr. Coons's laboratory in Boston and in Dr. Mitchison's laboratory in Edinburgh, where Dr. David Dresser, examining this very problem, has failed to reveal any clear distinction between the doses of soluble protein antigen needed to produce tolerance in newborn mice and paralysis in adults. I need not remind you, however, that no-one has yet produced any evidence that tolerance of homografts can be produced in adults merely by giving very high doses of those cells which, in lower doses, will produce tolerance in newborns. So far as cellular antigens are concerned, the empirical distinction between tolerance and paralysis remains valid.

I said that experiments designed to distinguish between tolerance and paralysis seem easy in principle, though they are very laborious in practice. There is however an important difficulty of principle. It is becoming very clear, particularly from Dresser's experiments, that 'antigen dosage' involves *two* variables—first the dosage necessary to *institute* the state of tolerance or paralysis, and second the dosage necessary to *maintain* it; and a suitable experimental design must take both variables into account. The work of Smith and Bridges and latterly of Dresser shows that the maintenance dose may be very low indeed—unresponsiveness is maintained by something like 10^{10} molecules of protein antigen—and this is a fact I shall refer to later in the lecture.

As a final remark to conclude the first part of this lecture, I feel bound to protest against the view that there is no such thing as immunological tolerance on the grounds that it is merely a form of immunological paralysis. Tolerance is a phenomenon that occurs naturally (as it does in cattle and chick twins, for example), and it *may* have a natural physiological role to perform in suppressing the action of potential auto-antigens. Dr. Hašek's and our own original experiments on tolerance may be regarded as an experimental reproduction of a phenomenon that occurs naturally in certain kinds of twins. Immunological paralysis, however, is a highly contrived laboratory artefact. I think then it would be an error of judgement to describe tolerance as a form of paralysis, though it may turn out that paralysis is a form of tolerance.

That concludes the first part of my lecture, on the empirical classification of immunologically unresponsive states, and you can see how very much more work must be done before we can be confident in our classifications.

II.

With some doubts and misgivings I should now like to discuss an entirely theoretical problem—the basis of tolerance and immunity in terms of cellular genetics; and I shall begin with the question first clearly formulated by the distinguished American geneticist, Joshua Lederberg: where does the information for antibody synthesis come from? What is the source of the instructions which determine that a gamma-globulin molecule, a molecule of antibody, shall be assembled in the particular specific way which is complementary to the structure of antigen? You will remember that Lederberg envisages two extreme possibilities in answer to this question—on the one hand an 'instructive' relationship between antigen and the responding cells, and on the other hand an 'elective' relationship.

According to the instructive theory (the theory which until recently we all believed in) it is the antigen itself that gives the instructions for the manufacture of specific antibody. It is the antigen that so to speak *teaches* the cell how to make a particular specific antibody, in much the same way, in principle, as a die-stamp or mould impresses its structure upon the material which takes its shape. This is the theory of antibody formation that lends itself most easily to a biochemical interpretation, and the theories of antibody formation associated with the names of Pauling, Mudd and Haurowitz are in fact 'instructive' theories. The information for making antibody comes from *outside* the cell: that is the point. Now if the instructive theory is true, then there is no theoretical reason to doubt that the antibody-forming cells within any one individual form a homogeneous population—and by 'homogeneous' I mean that each antibody-forming cell has the same potentialities: each cell *can* make any antibody within the repertoire of the organism as a whole, and the population is thus multipotent. To say that all cells have the same potentiality is by no means to say that all will have the same performance. A cell which has made an antibody against antigen A may thereafter be incapable of making an antibody against antigen B—but we assume that it *could* have made an anti-B antibody if antigen B had reached it first.

The other extreme possibility is an *elective* theory of antibody formation*. Here the information necessary for the manufacture of specific antibody is inside the cell. It is part of the genetic endowment of the cell, and the antigen simply releases, develops, brings out or exploits information which is already present within the reacting cell. The antigen is a trigger or releaser or inducer or evocator.

I should first say that the history of biology offers plenty of theoretical inducement to believe in an elective theory of antibody formation in spite of a certain almost instinctive resistance to it. In the history of biology it has happened several times that the reactions which we thought to be instructive in character turn out to be elective. In bacterial adaptation, for example, it used to be believed that the inducer of bacterial adaptation was an instructive stimulus; in fact, in English we describe the adaptive process as the "training" of bacteria—which means educating or instructing them; but it is now known that the inducer of bacterial adaptation is an elective stimulus which

*) Cp. Lederberg, J.: J. Cell. Comp. Physiol. (Suppl. 1), *52* : 398, 1958; Science, *129* : 1649, 1959. Burnet, F. M.: *The clonal selection theory of acquired immunity*. Cambridge 1959.

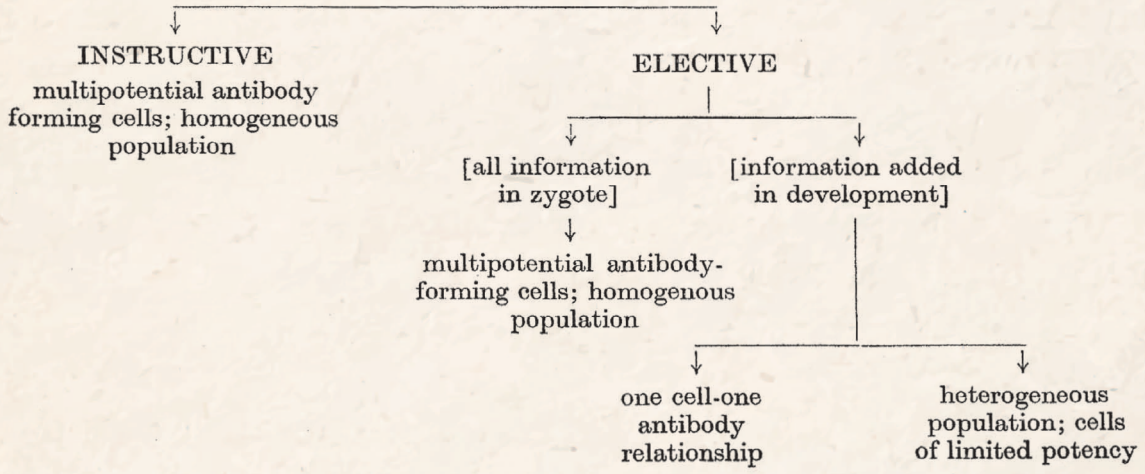
merely brings out or releases an inhibition of some genetic potentiality already present within the bacterial cell. At one time people spoke of the 'organizer' in embryonic development, and it used to be believed that the organizer was a source of instruction in the technical sense we have in mind; but I think that most embryologists now agree that the organizer is an elective or evocative principle which develops some potentiality present in the responding system. A third example comes from population genetics and evolution theory; according to the doctrine rather vaguely known as 'Lamarckism' the environment can actually impress genetical instructions upon an individual animal and so alter the genetic character of its offspring; but most of us are quite satisfied that the mechanism is an elective one; that is to say the environment can develop or bring out one genotype rather than another; but it cannot alter an individual's genotype in any specific way.

There is, then, some theoretical inducement to believe that the mechanism of antibody formation is elective in character. But now a difficulty arises. Microbiologists like Burnet, Monod and Lederberg find it difficult to believe that it is physically possible for the zygote, the fertilized egg, to contain all the instructions necessary for making every kind of antibody which the adult animal is capable of forming: they are inclined to think, therefore, that *new* genetical information is added in the course of development (the addition of new genetic information is, by definition, 'mutation'). Mutations must therefore occur in that lineage of cells descending from the zygote which eventually gives rise to the population of antibody-forming cells in the adult, and these mutations enlarge the repertoire of immunological response.

Now this is not necessarily or self-evidently true. Perhaps the zygote *does* contain all the information necessary to subsidize the formation of antibodies. After all, the zygote contains enough genetical information to subsidize the formation of an anatomically and physiologically very complex adult animal with complex behaviour; why should it not also contain the information necessary to guarantee the formation of 200 or 2000 different kinds of antibodies?

So there are two forms of the elective theory (fig. 1). According to one possible variant, no new information is added and no mutations occur in the lineage of antibody-forming cells descending from the zygote. If this is so, then antibody-forming cells will be a homogeneous (i.e. a genetically similar or isogenic) population, and each cell will be multipotent in the sense I explained when speaking of the instructive theory. If this form of the elective theory is true, it will be

Fig. 1.



extremely difficult to distinguish empirically between an instructive and an elective theory. But, as I say, our microbiological colleagues are satisfied that there is *not* enough information in the zygote to support the immunological repertoire of the adult, and in the alternative form of the elective theory, mutations occur in the lineage of antibody-forming cells and new information is added. This form of the elective theory may again be subdivided into two variants. According to Burnet, mutation occurs during development in such a way that the antibody-forming cells form an assemblage of clones, each one of which is competent to make only one kind of antibody. Needless to say this interpretation does not logically follow from the elective theory; as we shall see, it is a special theory put forward by Burnet to explain the phenomenon of immunological tolerance.

Monod has pointed out that it is by no means necessary to assume that mutations occur in such a way that any one cell becomes genetically competent to make only one antibody. Plural mutations may occur in such a way that one cell can make more than one antibody, but not every kind of antibody. It may make two or three different kinds of antibody or perhaps 20, but there is no need to assume the existence of a one cell-one antibody relation. Monod's suggestion thus points to a less extreme form of elective theory than the theory proposed by Burnet.

Now the problem of whether or not one cell can make more than one antibody is a problem of very considerable practical importance. You will remember that Lederberg, when he was working in Burnet's laboratory in Melbourne in collaboration with Dr. Nossal, produced evidence from the study of Salmonella antigens that most cells did in fact make only one antibody. Since then some careful work by Dr. Melvin Cohn and Dr. Lennox has shown quite clearly that in some immunological systems (they were using bacteriophage antigens) one cell can make two antibodies. But I do emphasize that in spite of its great empirical importance, whether or not one cell makes more than one antibody is not *theoretically* decisive for any theory of antibody formation. As I have said, the acceptance of an elective theory of antibody formation does not logically necessitate accepting the one cell-one antibody relation. Conversely, even if it should be true that one cell can make only one antibody, this would not logically necessitate the acceptance of an elective interpretation. It is quite compatible with an instructive theory; as I said a moment ago, a cell which was potentially capable of making antibodies against antigens A and B might be unable to make anti-B if antigen A reached it first or anti-A if antigen B reached it first; the first antigen to reach the cell might occupy the whole of its synthetic machinery for some time to come. Why then has Burnet attached particular importance to the one cell-one antibody concept?

The reason is that it provides a possible explanation of tolerance, which runs as follows. At a certain early stage in its development or maturation a future antibody-forming cell is hypersensitive to the action of antigen; at this stage exposure of the immature cell to antigen will kill it. But if the cell population is subdivided into different clones, each one capable of making only one antibody, then exposure to antigen will eliminate the clone by killing all the cells which are capable of making the particular antibody that corresponds to the antigen the cells were exposed to. So tolerance is produced: tolerance is the state that results from the actual elimination of the cells genetically predetermined to make a particular kind of antibody. This view has interesting theoretical consequences; it implies, for example, that one can only speak of a tolerant *animal* and never of a tolerant cell, for according to this theory a tolerant cell is dead, a cell that no longer exists. For this reason it is

very important to try to devise an experiment to find out if there really is such a thing as a tolerant cell. It sounds easy, but in practice it is very difficult to think of an experiment to distinguish between the presence of a tolerant cell and the absence of a non-tolerant cell; perhaps Dr. Simonsen's test system can be adapted to the purpose.

There is another difficulty about this theory of tolerance. If tolerance involves the killing and elimination of a particular clone of cells, why should it be necessary for the antigenic stimulus to be maintained in order to maintain the state of tolerance? The tolerance produced in a rabbit by the injection at birth of bovine protein is known to disappear unless the injections of bovine protein are maintained through the animal's life. It is true that only very small quantities of antigen, perhaps 10^{10} molecules, are necessary to maintain the state of tolerance—but why should *any* antigen be necessary to maintain tolerance if tolerance consists of the elimination of reactive cells?

To get over this difficulty, Lederberg suggests that antibody-forming cells arise from immature stem cells throughout the animal's life, and that in course of maturing they pass through a state in which they are vulnerable to the action of antigen. This then is the reason why antigen must persist to maintain the state of tolerance: the antigen is needed to produce tolerance in respect of these new, immature cells which are being added to the antibody-cell population throughout life.

In a quantitative sense this argument is not very satisfactory. If it is indeed true that only something like 10^{10} molecules of protein antigen are necessary to maintain a state of tolerance then the argument implies that very much lower concentrations of antigen are needed to produce tolerance in these newly formed, newly differentiating antibody-forming cells than are needed to produce tolerance in embryos, in which the entire antibody-cell population is immature.

So I am not satisfied, at the moment anyhow, with Burnet's and Lederberg's interpretation of the phenomenon of tolerance. But of course it may still be true that antibody-forming cells do mature throughout life, i.e. that there is a constant recruitment of new cells to the antibody-forming population. This theory was, I think, first proposed by John Loutit, the Director of the Medical Research Council's Radiobiological Research Unit at Harwell, as a possible explanation of the phenomenon of radiation induced tolerance. His conception was that when an animal is irradiated, the mature and fully formed antibody-forming cells are destroyed, leaving only immature cells upon which the action of antigen is to produce tolerance. This theory may be correct, but there is at present no critical evidence in its favour.

I may end by just summarising the main points I should like to make in a purely personal statement of opinion. I myself am inclined—more on general grounds than for any exactly justifiable reason—to believe in an elective theory of antibody formation. I think it is biologically more plausible—it fits better with our general conception of biological reactivity. I am not yet convinced, however, by the argument that genetic information must be added to in the course of development, i.e. that the zygote cannot possibly contain enough information to subsidize the formation of all the antibodies an adult is capable of making. I should also like to emphasize that although the problem of whether or not one cell can make more than one antibody is empirically very important, yet from the theoretical point of view the answer is not decisive for any theory of antibody formation.

Thirdly I am prepared to believe that in terms of cellular reactivity, tolerance and paralysis may be similar phenomena. But I still think it probable that the embryonic cell is more easily paralysed than the adult cell, or, if you like, that the antibody-forming cell in the adult

animal is more difficult to make tolerant than the immature cell which occurs in embryos, so that there is at least a quantitative distinction between the phenomena of paralysis and tolerance.

At present I also think that there probably is such a thing as a tolerant *cell*—that is, I am not quite satisfied with a theory which attributes tolerance to the killing of a cell and the elimination of the clone descending from it. The inception of tolerance probably represents some kind of cellular adaptation. Many years ago, Burnet suggested that an analogy could be drawn between antibody formation and adaptive enzyme formation in bacteria; but perhaps the analogy lies between immunological tolerance and adaptive enzyme formation in bacteria. But this is pure guesswork in a field in which there are already too many guesses, and I shall discuss the matter no further.