Cell-bound Antibodies

Conference of the
National Academy of Sciences–
National Research Council
Held May 10, 1963

Proceedings edited by
BERNARD AMOS AND HILARY KOPROWSKI

PUBLISHED JULY 15, 1963 BY THE WISTAR INSTITUTE PRESS

CELL-BOUND ANTIBODIES

Conference of the
National Academy of Sciences · National Research Council
Held May 10, 1963

Sponsored by
Committee on Tissue Transplantation of the
Division of Medical Sciences
National Academy of Sciences · National Research Council

Proceedings edited by
BERNARD AMOS AND HILARY KOPROWSKI

PUBLISHED JULY 1963 BY
THE WISTAR INSTITUTE PRESS · PHILADELPHIA

PUBLISHED JULY 1963

Library of Congress Catalog Card Number 63-20689

PRINTED IN THE UNITED STATES OF AMERICA AT
THE WISTAR INSTITUTE PRESS

COMMITTEE ON
TISSUE TRANSPLANTATION

NATIONAL ACADEMY OF SCIENCES — NATIONAL RESEARCH COUNCIL

DR. E. J. EICHWALD, *Chairman*
Montana Deaconess Hospital

DR. BERNARD AMOS, *Conference Chairman*
Duke University Medical Center

DR. CHARLES C. CONGDON
Oak Ridge National Laboratory

DR. RICHARD H. EGDAHL
Medical College of Virginia

DR. HILARY KOPROWSKI
The Wistar Institute

DR. H. SHERWOOD LAWRENCE
New York University Medical Center

DR. JOSEPH E. MURRAY
Peter Bent Brigham Hospital

DR. PAUL S. RUSSELL
Massachusetts General Hospital

DR. JOHN J. TRENTIN
Baylor University

DR. HENRY J. WINN
Roscoe B. Jackson Memorial Laboratory

CONTENTS

Supported by The National Institutes of Health,
Public Health Service Contract Number PH 43-62-167

Introduction to the Conference

E. J. EICHWALD
Montana Deaconess Hospital, Great Falls, Montana

THE study of many complex immunological reactions, such as delayed hypersensitivity, autoimmune diseases, and homograft rejection has been hindered because of the difficulty of reproducing the various conditions *in vitro*.

Within the three years preceding this conference, a number of long-awaited developments removed many of the difficulties. The demonstration of the cytotoxic property of lymphoid cells in tissue culture, characterization of a class of antibody with peculiar affinity for cells, the use of tritiated thymidine to trace the origin of cells entering into an immune reaction, and preliminary reports of the extraction from lymphoid cells of a factor, which would cause accelerated rejection of skin grafts, were examples of these developments.

Because these stimulating and still somewhat provocative discoveries had been made by investigators in different and to some extent unconnected fields, the Tissue Transplantation Committee of the National Academy of Sciences — National Research Council, acting upon a suggestion of Dr. Hilary Koprowski, planned a small, one-day conference at which the advances in technique and the changes in concept arising from the experimental work which followed, could be discussed at some length. The papers, which were read in outline, and most of the discussion which followed, are presented in these proceedings.

General Perspectives

(MORNING SESSION)

H. S. LAWRENCE

Department of Medicine, New York University Medical Center, New York, N. Y.

THE NOTION that cells engaged one way or another in certain immunological activities interact in a specific fashion with antigen is not a new one — and its evolution need not be detailed here. However, it may prove useful to select certain incidents that have led us to our current preoccupation with biological activities that qualify certain cell populations as effector reagents in immune responses.

One of the precipitating events has been the frustrating paradox of the ease with which serum antibodies are detected in a variety of altered tissue reactive states — such as delayed allergy, solid tissue homograft responses, allergic encephalomyelitis and thyroiditis — and the failure to implicate such antibodies as mediators of tissue destruction encountered in either actively or passively sensitized animals. Quite to the contrary, certain humoral antibodies have been rescued only recently from the status of an immunological irrelevancy by the findings of Kaliss on enhancement of tumor growth by antibody and Paterson's observations that humoral antibody protects animals otherwise destined to develop allergic encephalomyelitis.

The paradox of humoral antibody without discernible function led to the search for instruments of tissue damage in cells of the leucocyte series. The cellular transfer system pioneered by Landsteiner and Chase, and by Chase afforded the nearest semblance to an effector reagent for analysis of the immunological events leading to tissue destruction. The demonstrated effectiveness of this approach for delayed bacterial allergy soon led to its adaptation to the homograft problem, to allergic encephalomyelitis and to other experimental auto-immune disorders with considerable success.

Neglected, in its rapid application to a variety of altered tissue reactive states was Chase's early, important finding that depending on the mode of sensitization of donors, the cell populations

used may *simultaneously* transfer both delayed allergy and the capacity for serum antibody formation to the same antigenic determinant. This finding until recently, has not received the attention it deserves in assessing the mechanisms (e.g. delayed allergy vs. serum antibody) by which the transferred cells may produce the effects observed.

It may be of interest in this regard to consider the situation in humans where peripheral blood leucocytes or their extracts have been shown to transfer *only* delayed allergy and not the capacity for serum antibody formation, either to normal recipients or to agammaglobulinemic subjects. However, cell populations obtained from lymph node slices of normal humans have transferred both delayed allergy and the capacity to form serum antibody to agammaglobulinemic patients.

The latter finding approximates more nearly the experimental technic of many animal transfers. It is curious that the possibility of dissociating cell populations engaged in serum antibody production *from* those engaged in delayed allergy has not been exploited by the use of blood leucocytes in animal transfers. Chase showed blood leucocytes of guinea pigs were effective in the transfer of tuberculin allergy. More recently, Medawar and his colleagues observed blood leucocytes to be as effective as lymph node cells draining the grafted area in eliciting the "Transfer Reaction" to homograft antigens in guinea pigs.

This brings us to a critical matter, in speaking of cell-bound antibodies to designate *what* cells and *what* antibodies or immune functions are under consideration. It also allows for the sensible possibility of the existence of more than one type of cell-bound antibody. This has sound precedent in the work of unravelling more than one type of humoral antibody — an exercise which has been done with such exquisite precision over the years.

In this connection such considerations as the quality and quantity of antigen used, whether a single or repeated exposure is given, the route of access of antigen to the cell populations under study and the state of immunological reactivity of such cells at the time the specific questions are asked — will condition the nature of the answers secured. Attention to this sort of detail has aided the clear delineation of the varieties of antibody encountered in the serum and should prove of value in analysis of the varieties of cell-bound antibody.

Happily this process has already begun in the adaptation of information gained from *in vivo* events following cell transfer to

in vitro models. This transition has allowed dissection of discrete events and has led to documentation of an important concept that some of the cells involved in the reactions observed may be innocent bystanders enveloped by a humoral antibody they never made, other cells engaged full-tilt in fabricating and secreting antibody into their environs and still other cells synthesizing antibody but unable or unwilling to relinquish it. This leads one to question whether the cell or cells involved are functioning as *prime movers* in the tissue damage encountered or merely *convenient vehicles* for conservation and dissemination of minute quantities of otherwise undetectable humoral antibodies.

The *in vitro* analysis of cellular events has also documented a circumstance that until recently has been taken as an act of faith *in vivo* — namely that more than one cell type is involved in the labyrinthine route from afferent antigenic stimulus to efferent immune response. The findings of Fishman in respect of the obligatory need for processing antigen by macrophages before lymphoid cells can respond with the production of antibody is the case in point. This observation has shown the way to attempt analysis of the contributions of separate events in mixed cell populations engaged in tissue destruction. Moreover it promises to allow an *in vitro* approach to the study of the transfer of immunological information from cell to cell.

Just as Fishman's finding has indicated the deception of asking one cell type to express the versatility of a multicellular organism — other findings have pointed to the weakness of the unspoken assumption that every last cell of a particular population is sensitized or poised to accomplish the particular immunological feat requested by the experimentalist.

That this is not so has been intimated by recent reports from several laboratories demonstrating a paucity of labeled cells following transfer appear in the sites of delayed types of allergic inflammatory responses that have been transferred. This could be interpreted to mean either the number of sensitized cells engaged in launching events in tissue damage is small or there may occur a transfer of information between cells without regard to the location of the tritiated thymidine.

The work of David and Askari and colleagues has demonstrated that relatively few cells need to be sensitized. In an *in vitro* system designed to study effects of antigen on migration of macrophages from guinea pigs with delayed allergy, they have found as little as 2.5% of sensitized cells seeded into aliquots of 100%

normal cells, caused the entire population to behave as if obtained from a sensitized animal.

I would like to close with a consideration of a former candidate for the designation cell-bound antibody which it turns out is neither *cell-bound* nor *antibody*. Recent studies on the nature of transfer factor in human leucocyte extracts have revealed it to be a soluble, dialyzable material which is not a protein by chemical test nor a globulin fragment by immunological test. It resists lyophilization and ribonuclease treatment and is eluted from sephadex G-25 under a broad peak in the region where materials of $<$ 10,000 mol. weight are encountered. It is not an antibody in the conventional sense and the transferred sensitivity to bacterial, fungal or tissue homograft antigens surely not a passive transfer — again re-emphasizing the recipients' contribution to the events following transfer.

This has been, in extremely abbreviated form, a selective look at the scope of the problems surrounding the study of cell-bound antibodies. I have deliberately avoided anticipating the papers to be presented which contain, I am sure, answers to some of the queries posed. To do so would have been to distract and lessen the keen enjoyment we all feel in anticipation of the intellectual fare yet to come.

Cytophilic Antibody

STEPHEN V. BOYDEN
Department of Experimental Pathology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia

The term "cytophilic antibody" is used to describe a globulin component of immune serum which becomes attached *in vitro* to certain cells in such a way that these cells are subsequently capable of specifically adsorbing antigen. The existence of this antibody has been revealed with the aid of soluble antigens labeled with a radio-active isotope (Boyden and Sorkin, '60, '61). Cytophilic antibody is thus a substance which has distinct properties, and which can easily be demonstrated by a simple *in vitro* serological procedure; its name does not presuppose any particular *in vivo* function or significance.

The expression "cytophilic antibody" thus contrasts with more familiar terms such as "cellular," "cell-bound" and "cell-fixed antibody" which are generally used to describe substances which have not been clearly demonstrated either *in vitro* or *in vivo*, but which are considered to be responsible for a variety of unexplained immunological phenomena. In most cases the sole justification for the use of these expressions has been the fact that, while certain *in vivo* immunological reactions have characteristics in common with reactions known to be due to classical serum antibodies, investigations have failed to reveal the responsible substances in serum. It has therefore been taken for granted that these particular immunological reactions are due to antibodies which are associated with cells. Unfortunately, attempts to demonstrate them on or in cells have, in general, been no more successful than the attempts to demonstrate them in serum — a fact which the uninitiated would hardly guess from the frequency with which some authors refer to "cell-bound" antibodies as if they were distinct entities, the existence of which was well established. The fact that tuberculin-type hypersensitivity and homograft immunity can be passively transferred to normal animals by means of cells is no argument for the involvement of cell-bound antibodies, since anaphylaxis (Chase, '54; Stankovic and Vlahovic, '61) and Arthus type hypersensitivity (Boyden, unpublished), which are certainly due to serum antibodies, can also be transferred with cells.

To say that the term cytophilic antibody presupposes no *in vivo* significance of this substance does not mean that there has been no speculation on this point. On the contrary, speculation has been rampant, and a series of experiments has been carried out aimed at revealing a special role for cytophilic antibody in one of several immunological phenomena. Until recently these experiments have yielded only negative results, but since negative results are not necessarily meaningless, I shall take the opportunity offered by this conference to summarize this work, some of which might otherwise never find its way into print. Some more encouraging results, obtained within the past few weeks, will also be described. Finally, I shall indulge in a page or two of speculative discussion on aspects of the problem of antibody production in relation especially to the possible role of cytophilic antibody or similar substances.

To begin with, let us summarize our knowledge about the general properties of cytophilic antibody and of its reaction with cells.

1. Normal spleen cells treated *in vitro* with cytophilic antibody, and then washed, are capable of specifically adsorbing the antigen. The uptake of antigen (Human Serum Albumin) per cm^3 of packed spleen cells, after treatment with a 50% dilution of a strong immune serum, may be in the order of 1–2 μg (Boyden and Sorkin, '60, '61).

2. Cytophilic antibody has been demonstrated in the serum of immunized rabbits against the following antigens.
   (a) Human Serum Albumin (HSA) (Boyden and Sorkin, '60).
   (b) Ovalbumin (Boyden and Sorkin, '60).
   (c) Timothy grass pollen (Sorkin, '63).
   (d) Diptheria toxoid (Sorkin, '63).
   (e) Human γ globulin (Sorkin, '63).
   (f) Horse Serum albumin (Sorkin '63).
3. It is not known which particular cells take up cytophilic antibody, although it seems probable that this property is shared by more than one cell type.
   Specific uptake of antigen has been demonstrated in the case of the following cell preparations after treatment with rabbit anti-HSA serum.
   (a) Rabbit and guinea pig spleen cells (Boyden and Sorkin, '60).
   (b) Cells in glycogen-induced exudates in guinea pigs and rabbits, containing in some cases predominately polymorphs, in others predominately macrophages. The uptake was similar in both cases, as it was in the case of suspensions from lymphoid tissue, comprising predominately lymphocytes (Boyden and Sorkin, unpublished).
   (c) Rat mast cells (Keller and Sorkin, '63).
   (d) Rat liver cells (Keller and Sorkin, '63).

   Spleen cells from the rabbit, horse, guinea pig, and rat have been shown to take up rabbit cytophilic antibody. Preparations of rabbit or guinea pig red cells did not take up appreciable amounts of antigen (Boyden and Sorkin, '60, '61).
4. In paper electrophoresis, preparative starch electrophoresis and free electrophoresis, cytophilic antibody has been found to have the mobility of γ 1 and γ 2 globulins. In the analytical ultracentrifuge it behaves as a 7S globulin (Sorkin, '63).
5. Absorption of anti-HSA serum with sufficient spleen cells to remove all detectable cytophilic antibody results in only a slight drop in the level of precipitating antibody in the serum (Boyden and Sorkin, '61). Calculations indicate that cytophilic antibody is responsible for only a small fraction (in the region of 0.1–1%) of the antigen-combining capacity of ordinary rabbit anti-protein sera.
6. Cytophilic antibody is not adsorbed by cells which have been treated at 56°C. Treatment of cells with methanol does not destroy, although it decreases the capacity of cells to take up cytophilic antibody.
   If cells which have adsorbed cytophilic antibody are heated at 56°C, the cytophilic antibody is eluted. Cytophilic antibody eluted in this way can render fresh cells capable of specifically binding antigen (Boyden and Sorkin, '60, '61). In such eluates the ratio of cytophilic activity to precipitating antibody is at least 100 times greater than in the original serum.
7. Treatment of spleen cells for short periods with trypsin and papain destroys completely their capacity to adsorb cytophilic antibody. Treatment with wheat germ lipase results in a partial decrease in capacity to adsorb the antibody (Sorkin, '63). These results have led Sorkin to suggest that the receptor on the cell surface is a lipo-protein.

These are the simple facts about cytophilic antibody. We may now turn to consider its possible significance in relation to (a) anaphylaxis, (b) chemotaxis, (c) delayed-type hypersensitivity and transplantation immunity, (d) antibody production.

(a) *Anaphylaxis.* While anaphylactic or immediate type hypersensitivity is transferable with serum, it is nevertheless usually supposed that anaphylaxis is the consequence of a reaction of antigen with cell-bound antibody. There is some evidence, perhaps not conclusive, to support this view (Dale, '12; Benaceraff and Kabat, '49), but there are no data showing whether or not the cell-associated antibody actually binds the antigen to the cell.

Although the prevailing opinion at present is that ordinary precipitating antibody

renders tissue anaphylactically sensitive to antigen, the possibility cannot be excluded that anaphylaxis is due to a reaction between antigen and a special type of antibody with a strong affinity for cells. The question naturally arises whether cytophilic antibody could be the responsible substance.

Experiments on this question have given results which strongly suggest that cytophilic antibody is of no special significance in relation to anaphylaxis. Solutions of cytophilic antibody, prepared by elution from cells at 56°C, have been compared with original serum for capacity to sensitize guinea pig skin in passive cutaneous anaphylaxis (P.C.A.). While the cytophilic activity of such eluates was only slightly less than that of the original serum, their reactivity in the P.C.A. test was 100–1,000 fold less (Sorkin and Boyden, unpublished results).

Sorkin has compared the P.C.A. activity and cytophilic antibody activity of γ globulin fractions of an antiserum eluted from a DEAE cellulose column. He found no correlation between the two activities. Indeed some fractions with high levels of cytophilic antibody showed no P.C.A. activity at all, a finding which suggests that cytophilic antibody is not capable of rendering the skin anaphylactically sensitive to antigen.

Similarly, results of experiments with the Schultz-Dale technique provided no evidence that cytophilic antibody plays any part in sensitizing smooth muscle to anaphylactic contraction on contact with antigen (Sorkin, personal communication). The conclusion that cytophilic antibody, defined as an antibody which is adsorbed by cells and which subsequently binds antigen to these cells, plays no part in anaphylaxis has interesting implications in relation to the mechanism of anaphylaxis; for it seems to indicate that the antibodies responsible for the anaphylactic reactions do *not* bind antigen to cells, at least not to cells of the types predominating in spleen tissue.

(b) *Chemotaxis.* It has long been known that polymorpho-nuclear leucocytes migrate actively both *in vivo* and *in vitro* towards bacteria, and that this response is intensified in the immunized animal (Metchnikoff, '05).

The possibility occurred to us that cytophilic antibody might play a role in enhancing the chemotactic response of leucocytes towards antigens diffusing from micro-organisms. Experiments were set up, using a technique recently described (Boyden, '62a), to test whether leucocytes coated with cytophilic antibody would show a greater chemotactic response than normal leucocytes to a concentration gradient of soluble antigen (HSA). No difference was observed between the migration of the normal and of the antibody-coated leucocytes.

The finding that antibody-antigen complexes activate normal serum components with the consequent production of a chemotactic principle, which acts directly on polymorpho-nuclear leucocytes, provides an alternative explanation for the increased migration of leucocytes towards bacteria in an immunized animal (Boyden, '62a).

(c) *Delayed-type hypersensitivity and transplantation immunity.* The term "cell-bound antibody," and similar expressions, have probably been used more in relation to tuberculin-type hypersensitivity and transplantation immunity than in any other connection.

Following the initial demonstration of cytophilic antibody in rabbit antiserum to HSA, experiments were immediately set up aimed at the detection in the serum of tuberculous guinea pigs of similar substances which would render cells from normal guinea pigs capable of specifically adsorbing protein antigens of the tubercle bacillus. Sera taken from guinea pigs which were strongly tuberculin sensitive were tested by the same technique as used in the rabbit anti-HSA system: none showed any sign of cytophilic antibody to tuberculo-proteins. This disappointing result was consistent with our repeated failures to demonstrate a difference between the uptake of labeled tuberculo-proteins by cells taken from normal and from tuberculin-sensitive guinea pigs. Turk ('60), however, has reported a slightly greater uptake of radio-active tuberculin by lymphoid cells of tuberculin-sensitive guinea pigs.

One of the difficulties encountered with preparations of mycobacterial antigens in this kind of study is the fact that normal cells take up a considerable amount of the antigen, and it is possible that this "non-specific" adsorption masks any specific uptake. For this reason, it would be advantageous to work with an antigen, such as HSA or BSA, which shows relatively little adsorption onto normal cells. Turk ('60) has reported that lymphoid cells from animals with delayed-type hypersensitivity to BSA, induced by immunization with picrylated BSA according to the method of Gell and Benaceraff ('59), take up considerably more labeled BSA than do cells from normal animals. Unfortunately, we have not succeeded in producing strong "pure" delayed type hypersensitivity to BSA by this means, and so we have not had an opportunity to confirm this result. We have succeeded in inducing strong delayed-type hypersensitivity to HSA and to BSA by injecting the antigen into the footpad with Freund's complete adjuvant — according to the principle of Dienes (for references see Boyden, '58), who observed that delayed reactivity to ovalbumin developed following the injection of the antigen into tuberculous lesions. At 24 and 48 hours the skin reactions to HSA or BSA in animals immunized in this way look very like pure tuberculin reactions, showing marked induration, often with a central area of necrosis. However, unlike the situation in tuberculin-type allergy induced by living bacilli, there is also a marked Arthus component to the reaction, so that there is considerable edematous swelling of the skin at 3–6 hours after the intradermal injection of antigen (Nelson and Boyden, '63a). We have attempted to demonstrate a specific adsorption of radio-active labeled antigens by lymphocytes and macrophages from guinea pigs immunized in this way, but so far we have obtained very inconsistent results, the interpretation of which is not at all clear at present.

More interesting are some recent experiments (Boyden, unpublished) in which we immunized guinea pigs by injecting a 30% suspension of sheep erythrocytes with Freund's complete adjuvant into the foot-pads. When skin tested two or three weeks later with saline or urea extracts of sheep erythrocytes, these animals show strong "pure" tuberculin-type reactions with no detectable Arthus component. At 24, 48 and even 72 hours there is very marked induration with erythema and frequently a central area of necrosis. Cell suspensions, containing mostly macrophages and lymphocytes, but also varying amounts of polymorphs (up to 30% in some cases) were obtained by washing out the peritoneal cavity with Hanks solution containing heparin. The cells were washed four times with Hanks solution and then mixed in Hanks solution with a 0.5% suspension of sheep erythrocytes. The tube containing this mixture was left at room temperature for three hours, and then left in the cold room at 0–4°C for 8 or 9 hours. The cells were then agitated gently and examined microscopically.

In the case of preparations from the immunized animals, 30–70% of the macrophages and lymphocytes had five or more erythrocytes adherent to their surfaces. Often the number of adherent erythrocytes was sufficient to produce a characteristic "rosette" appearance. In contrast, only occasional cells from normal animals were attached to erythrocytes, and the proportion with five or more erythrocytes attached was 0%. It is important that this test be carried out in the absence of serum.

When peritoneal cells from normal guinea pigs were treated for one hour in the cold with serum from the immunized animals, washed four times, and then mixed with sheep erythrocytes, they reacted with the latter in the same manner as cells from the immunized animals. Similar treatment with normal serum *in vitro* or with serum of animals immunized with HSA and Freund's adjuvant did not render normal peritoneal cells reactive.

The factor in the immune serum which confers on normal peritoneal cells an affinity for sheep erythrocytes conforms to our definition of cytophilic antibody. This cytophilic antibody, like that described in rabbit antiserum, is not destroyed by heating for thirty minutes at 56°C.

Possibly connected with this observation is the interesting report of Koprowski and Fernandes ('62), who injected rats with

guinea pig cord tissue and Freund's complete adjuvant, and showed that lymphocytes from the immunized animals showed a marked tendency to adhere to glial elements in tissue cultures of puppy brain. Similar properties could be conferred on lymphocytes from normal rats by pretreatment with serum from immune animals. It may well be that the specific agent in the serum responsible for this effect corresponds to cytophilic antibody.

These observations may also have some connection with the finding that the intravenous or intraperitoneal injection of antigen causes the apparent disappearance within an hour or so of macrophages from the peritoneal cavities of guinea pigs with tuberculin-type hypersensitivity (Nelson and Boyden, '63b). Although the mechanism of this effect is not yet understood, it is thought that it is due to clumping of the macrophages and their adherence to the walls of the peritoneal cavity.

We can conclude from these various experimental findings only that the possibility cannot be ruled out at present that cytophilic antibody may play a part in delayed-type hypersensitivity or in transplantation immunity. More work is necessary, and among other things, it would be worth looking into the possibility of a connection between cytophilic antibody and Lawrence's "transfer factor" (Lawrence, '59).

(d) *Antibody production.* It is customary these days for authors to discuss almost any immunological finding in terms of their favorite theory of antibody production which, at the present time, is usually the clonal selection theory (Talmage, '57; Burnet, '57, '59; Lederberg, '59). I will now proceed to follow this custom with respect to observations on cytophilic antibody. I will not, however, discuss cytophilic antibody in terms of the clonal selection theory, since, for reasons which have been set out elsewhere (Boyden, '60, '62b, '63), I consider it rather unlikely that the capacity of an animal to produce large numbers of different antibodies is based on genetic differences between potential antibody-producing cells. The clones of antibody producing cells which appear following antigenic stimulation have more likely arisen from genetically similar precursors which are multipotential with respect to the specificity of the antigens to which they are initially capable of responding. Once stimulated by a given antigenic determinant, such cells may well be capable of producing antibodies only against that single determinant.

Although there are some aspects of Jerne's natural selection theory (Jerne, '55) which are hard to accept, this theory is, in my opinion, likely to be nearer the truth than the clonal selection theory, particularly with regard to the early phases of antibody production, and the "recognition" of foreign antigens.

In this paper the discussion will be restricted to the problem of the capacity of the immune system to recognize foreign matter, a problem which, as Burnet (Burnet and Fenner, '49; Burnet, '59) has frequently pointed out, is a very crucial one in relation to our attempts to understand antibody production. Our own point of view is based on the fact that there is, even in the simplest multicellular animals, a mechanism for discriminating between foreign matter and healthy cells of the host, so that the individual's phagocytic cells attack the former but not the latter. Although the nature of this discriminatory process in primitive animals is not at present understood, we know that it is very effective, and it seems reasonable to suppose that the same basic mechanism may be responsible for the discriminative behavior of the cellular system responsible for antibody production.

Elsewhere (Boyden, '60, '62b, '63) it has been suggested that, in invertebrates, special molecules, which we may call "recognition factors," are produced and that these molecules, like antibodies, can exist in a wide variety of forms differing from each other in their specific affinities. Such molecules are liberated into the body fluids and can, by combining with groups on the surfaces of particles with which they happen to be reactive, act as opsonins, rendering the particles attractive to phagocytes. Molecules which happen to have an affinity for exposed self-components are quickly absorbed out of the body fluids; they are ineffective in promoting opsonization of host cells, since the num-

ber of molecules adsorbed onto any given host cell at any one moment is likely to be very small. In contrast, recognition factors or opsonins which have no affinity for normally exposed host components, will tend to accumulate either free in the body fluids, or possibly, like cytophilic antibodies, adsorbed to phagocytes. Thus, when a bacterium or similar foreign particle enters the tissues, it will be exposed to relatively high levels of specific opsonins, sufficient to render it susceptible to phagocytic attack. The phagocyte thus depends on the opsonins for its apparent capacity to recognize foreign matter. Unfortunately there are not at present enough data to show whether discrimination by invertebrate phagocytes is, in fact, based on this hypothetical mechanism. On the other hand experimental evidence strongly supports the view that phagocytic discrimination in vertebrates is dependent largely, if not entirely, on the presence in the circulation of opsonins or "recognition factors" reactive with foreign material (see review by Boyden, '63).

According to this hypothesis, there will occur an accumulation in the body fluids not only of opsonins reactive with foreign matter, but also of opsonins which happen to have an affinity for self components which occur intracellularly and which are not normally exposed to the body fluids. Accumulation of opsonins reactive with such hidden or inaccessible host components could account for the rapid uptake by phagocytes of damaged and dead host cells which occurs in both vertebrates and invertebrates.

The suggestion, then, is that discrimination by the antibody-forming system is similarly based on the production of recognition factors reactive against all manner of macromolecular groupings, and on the accumulation in the circulation to effective levels only of those recognition factors which have no affinity for normally exposed autogenous components. Two variants of this idea can be envisaged: (1) That the recognition factors of the immune system which are not reactive with exposed self-components accumulate and, like natural opsonins, remain free in the circulation. When a foreign antigen is given, complexes are formed which are then removed from the circulation by appropriate cells (cf. Jerne, '55). The recognition factors of the immune system and natural opsonins may be one and the same thing. (2) That the recognition factors of the immune system possess certain of the properties of cytophilic antibody, so that they can be adsorbed and rather firmly bound by cells without losing their specific affinity for certain macromolecular determinant groups. If such "cytophilic recognition factors" were produced at a steady rate, those of them which were reactive with exposed autogenous components would be quickly neutralized and effectively removed from the circulation. Recognition factors which were not reactive with exposed determinants would tend to accumulate, not free in the circulation, but because of their cytophilic properties, on the surfaces of cells. Among these cells would be the potential antibody producers. On entry of the antigen into the tissues, a sufficient number of potential antibody producing cells would be coated with recognition factors reactive with the particular unfamiliar determinant group to initiate an immune response. The capacity of an animal to respond to a given antigen would be a reflection of the level of accumulated recognition factors in that animal against the antigen at the time of antigenic stimulation.

If either of these two possibilities were correct, there would occur an accumulation of recognition factors, free or cell-fixed, not only against foreign groups but also against autogenous determinant groups normally hidden inside cells. Thus cell damage with release of macromolecules bearing such unfamiliar determinants into the circulation would be expected to result in antibody production. That this does in fact occur is borne out by the increasing number of reports of autoantibodies in sera of patients with various disease conditions involving extensive cell damage. Immunological tolerance is not an all-or-none phenomenon. There seems to be a fairly constant inverse relationship between an individual's capacity to produce antibodies to a given determinant group and the normal level, or rate of occurrence, of this group in the

circulation. Thus antibody production is maximal against antigens which never occur normally exposed in the body fluids, and minimal against substances which are normally present there. With sufficiently sensitive techniques all adult animals can probably be shown to have autoantibodies in the serum (Boyden, '63b), although the antibodies to intracellular components which are fairly frequently liberated into the circulation (e.g. following damage to skin epithelium) never reach very high levels in the normal animal.

To return to cytophilic antibody, is it possible that this substance is, in fact, the hypothetical "cytophilic recognition factor"? What little experimental evidence there is on this point provides no support for the view. We have tried unsuccessfully to increase the antibody response of both adult and newborn rabbits to HSA by injecting, before antigen, cytophilic antibody eluted from cells by treatment at 56°C (Sorkin and Boyden, unpublished). However these experiments have been on a small scale and cannot be regarded as conclusive.

There are, however, other reasons for suspecting that cytophilic antibody is not the postulated recognition factor, the most important of which is the fact that the level of cytophilic antibody in the serum increases during immunization. It is implicit in the recognition factor hypothesis that, although certain abnormal procedures, such as the injection of Freund's complete adjuvant may cause an overproduction of recognition factors, normally they are produced at a constant rate, even after contact with antigen. This supposition is necessary, for otherwise the constant release of very small amounts of intracellular determinant groups would stimulate the production of the corresponding specific recognition factors, thus eventually increasing the responsiveness of the antibody forming system to these antigens.

An alternative possibility is that the recognition factors are not cytophilic, but are free in the circulation, and are possibly identical with natural opsonins. This view of the discriminatory mechanism of the immune system is very close to Jerne's explanation of the lack of antibody production against normal self components (Jerne, '55). But there is one important difference; in Jerne's theory the recognizing substance is antibody, which combines with antigen, and after uptake by cells, begins a process of self-replication with consequent production of more of the same antibody. For reasons mentioned above, the postulated recognition factor is distinct from antibody, although it carries the same serological specificity.

The present paucity of definite data about cytophilic antibody is offered as an excuse for so much space in this paper having been given up to mere speculation. Time will show whether, in fact, cytophilic antibody plays a role in any of the immunological reactions in which its participation has been postulated. Perhaps the possibility most worthy of further investigation is that cytophilic antibody might be the "cell-bound" antibody which, although undetected *in vitro*, has for so long been held responsible for tuberculin-type hypersensitivity and, by some, for homograft rejection.

LITERATURE CITED

Benacerraf, H., and E. A. Kabat 1949 A quantitative study of passive anaphylaxis in the guinea pig. v. The latent period of passive anaphylaxis in its relation to the dose of rabbit anti-ovalbumin. J. Immunol., *62:* 517–522.

Boyden, S. V. 1958 The immunological response to antigens of the tubercle bacillus. Progr. Allergy, *5:* 149–214.

——— 1960 Antibody production. Nature, *185:* 724–726.

——— 1962a The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. Exp. med., *115:* 453–466.

——— 1962b Cellular discrimination between indigenous and foreign matter. J. Theoret. Biol., *3:* 123–131.

——— 1963a Cellular recognition of foreign matter. Internat. Rev. Exper. Pathology, *2:* (in press).

——— 1963b Autoimmunity and inflammation. Submitted for publication.

Boyden, S. V., and E. Sorkin 1960 The adsorption of antibody and antigen by spleen cells *in vitro*. Immunology, *3:* 272–283.

——— 1961 The adsorption of antibody and antigen by spleen cells *in vitro*. Some further experiments. Immunology, *4:* 244–252.

Burnet, F. M. 1957 A modification of Jerne's theory of antibody production using the concept of clonal selection. Austral. J. Science, *20:* 67–69.