This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 315

IMMUNOLOGY
AND PARASITIC DISEASES

Report of a WHO Expert Committee

Ibadan, 8-15 December 1964

WORLD HEALTH ORGANIZATION
GENEVA
1965
WHO EXPERT COMMITTEE ON IMMUNOLOGY AND PARASITIC DISEASES

Ibadan, 8-15 December 1964

Members:

Dr D. A. L. Davies, McIndoe Memorial Research Unit, Blind Laboratories, Queen Victoria Hospital, East Grinstead, Sussex, England (Rapporteur)
Professor F. J. Dixon, Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Cal., USA
Professor J. C. Edozien, Dean, University of Ibadan Medical School, Nigeria (Chairman)
Dr J. H. Humphrey, Head, Division of Immunology, National Institute for Medical Research, Mill Hill, London, England (Vice-Chairman)
Professor I. McIntyre, Dean, Faculty of Veterinary Science, University College, Nairobi, Kenya, and Professor of Veterinary Medicine, University of Glasgow, Scotland
Dr I. Ribn, Institute of Microbiology, Czechoslovak Academy of Science, Prague, Czechoslovakia
Professor W. P. Rogers, Department of Zoology, University of Adelaide, Australia
Professor E. J. L. Soulsby, Laboratory of Parasitology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa., USA

Secretariat:

Dr N. Ansari, Chief, Parasitic Diseases, WHO, Geneva (Joint Secretary)
Professor A. E. Boyo, Department of Pathology, Lagos University Medical School, Nigeria (Consultant)
Dr H. C. Goodman, Chief, Immunology, WHO, Geneva (Joint Secretary)
Professor E. A. Kabat, Department of Microbiology, Columbia University, New York, USA (Consultant)
Dr J. Kagan, Chief, Parasitology Unit, Communicable Disease Center, Atlanta, Ga., USA (Consultant)
Dr W. H. R. Lumsden, Bacteriology Department, University of Edinburgh Medical School, Scotland (Consultant)
Professor R. Masseyeff, Laboratoire de Biochimie médicale, Université de Dakar, Sénégal (Consultant)
Dr I. A. McGregor, Director, Medical Research Council Laboratories, Gambia, West Africa (Consultant)
Dr E. H. Sadun, Chief, Department of Medical Zoology, Walter Reed Army Institute of Research, Washington, D.C., USA (Consultant)
Dr P. P. Weinstein, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md., USA (Consultant)

© World Health Organization 1965

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. Nevertheless governmental agencies or learned and professional societies may reproduce data or excerpts or illustrations from them without requesting an authorization from the World Health Organization.

For rights of reproduction or translation of WHO publications in toto, application should be made to the Division of Editorial and Reference Services, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

PRINTED IN SWITZERLAND
CONTENTS

Introduction .................................................................................. 5

1. Basic developments in immunology ....................................... 6
   1.1 Antigens and antigenic determinants .................................. 6
   1.2 Heterogeneity of antibodies ............................................. 11
   1.3 Cytolytic and cytotoxic effects of antibody ......................... 16
   1.4 Pathology of antigen-antibody complexes ......................... 19
   1.5 Delayed-type hypersensitivity .......................................... 23

2. Characteristics of zooparasitic infections ............................... 24
   2.1 Life cycles of zooparasites ............................................... 25
   2.2 Factors influencing infection, development and the course of
       migration in the host ..................................................... 26

3. Some characteristics of immunity in parasitic diseases .......... 28
   3.1 Natural resistance ....................................................... 29
   3.2 Active and passive immunity .......................................... 30
   3.3 Immune mechanisms in invertebrates ............................... 39

4. Host responses implicated in immunity ................................. 41
   4.1 Specific effects on parasite ............................................. 41
   4.2 Hypersensitivity ......................................................... 43

5. Antigens of parasites ............................................................ 47
   5.1 Liberation during invasion of the host ............................... 47
   5.2 Relation to parasitic mass, physiology and reproduction ...... 48
   5.3 Antigenic variation ....................................................... 49
   5.4 Shared antigens ......................................................... 50
   5.5 In vitro cultivation and production of antigens useful for immu-
       nization ................................................................. 51

6. Methods .................................................................................. 52
   6.1 Immunodiagnostic techniques ........................................ 52
   6.2 In vitro methods for the detection of hypersensitivity .......... 55
   6.3 Standardization of inoculum and challenge dose ............... 56
   6.4 Action of antibody on parasites in vitro ........................... 57
   6.5 The use of germ-free animals ......................................... 58

7. Recommendations ................................................................. 59

— 3 —
IMMUNOLOGY AND PARASITIC DISEASES

Report of a WHO Expert Committee

The WHO Expert Committee on Immunology and Parasitic Diseases met in Ibadan, Nigeria, from 8 to 15 December, 1964. Dr L. Bernard, Personal Representative of the Director-General, WHO Regional Office for Africa, welcomed the members on behalf of the Director-General. He pointed out that recent advances in both the theoretical and the applied aspects of immunology make it more than ever necessary to stimulate immunologists and parasitologists to an increasing awareness of each other’s disciplines for the benefit of medical practice and public health problems. The Committee was therefore being asked to review recent advances in immunology that might be applicable to the parasitic diseases and, in addition, to indicate which studies of parasites and parasitic diseases might be potentially useful as models for investigating basic problems in immunology. One of the Committee’s tasks was to identify the gaps in present knowledge of the immunology of parasitic diseases and to suggest means of stimulating more study by immunologists of the fundamental problems of resistance to protozoan and metazoan parasites.

Dr J. C. Edozien was elected Chairman, Dr J. Humphrey Vice-Chairman, and Dr D. A. L. Davies Rapporteur.

INTRODUCTION

The close contact between immunology and bacteriology since the beginning of the century has not been paralleled by similarly close contact between immunology and parasitology. New knowledge is rapidly becoming available on the nature of antigens, the heterogeneity of antibodies, and the consequences of antigen-antibody interactions both in protecting the host and in damaging host tissue during hypersensitivity reactions. The need for bringing this new knowledge in immunology to bear on the problems of parasitic diseases was stressed in a report of five scientific groups on research in immunology, which summarized the present state of knowledge in immunology and laid the basis for the newly established WHO programme for research in immunology.¹

The scope of the subject of immunology in the many parasitic diseases is so vast that many areas could not be covered in the present report. None the less, the Committee thinks it advisable to attempt to deal with certain aspects of research in immunology in some detail, in order to provide more precise guidelines for the development of future areas of research in the parasitic diseases. The Committee has also attempted to delineate certain areas where it appears that research developments in immunology have applications to diagnosis, pathogenesis, and immunoprophylaxis in the parasitic diseases. Much use has been made of published reviews and reports of previous symposia and international meetings on various aspects of immunology and parasitic diseases.

It is hoped that the report, even though it discloses the wide gaps in present knowledge of the immunology of certain parasitic diseases, will nevertheless serve to provide immunologists with an outline of some of the problems with which parasitologists are faced, and induce more parasitologists to consider the possibility of a fruitful approach to these problems in terms of immunological concepts.

1. BASIC DEVELOPMENTS IN IMMUNOLOGY

Immunological concepts in certain areas have been considerably clarified in recent years. Selected aspects of some of these are presented in summary form in the following section, since they illustrate how these concepts may be applicable to the immunology of parasitic diseases.

1.1 Antigens and antigenic determinants

Antigens may be classified by their chemical nature. Antibodies against nucleic acids occur naturally in some disease states, such as lupus erythematosus, although it is only recently and under special circumstances that they have been produced experimentally in laboratory animals. A recent study reports the production of antibodies to nucleic acids by injecting mixtures of nucleic acid and methylated albumin, and several laboratories have succeeded in attaching purine and pyrimidine residues to proteins so that these products are antigenic and give rise to antibodies specific for the

---

purine or pyrimidine moiety. The latter antibodies have been shown to inhibit development of fertilized sea urchin eggs, and the possible effect of such antibodies on multiplication of parasites deserves close attention.

Polysaccharides and carbohydrate residues attached to protein (glycoproteins, mucopolysaccharides), to peptides (glycopeptides, mucopolysaccharides) or to lipids (glycolipids) possess antigenic determinants due to the sugar residues, especially those located at the non-reducing ends of polysaccharide chains. This is a field of study that has been very rewarding in the case of Pneumococcus capsular polysaccharides, Salmonella O antigens and human blood group mucopolysaccharides. Findings in this area may have value in immunoparasitology; for example, in an understanding of the basis for cross reactions. Although as many different sugar residues occur in immunologically specific polysaccharides as there are amino acids in proteins, only a small number are found together in any one polymer and the secondary structure is perhaps infrequently involved in determining specificity. It is therefore common to find cross reactions between carbohydrate-containing macromolecules from widely different animal and plant species, for example, from certain bacteria and man.

Salmonella O antigens are examples of the kinds of structures that endow carbohydrates with immunological specificity. Salmonellae can be classified into a number of groups (A, B, C, ...), members of each group differing from one another by the possession in their cell walls of several "antigens" (1, 2, 3, ...), at least one of which is characteristic of the group. Thus group B (e.g., Salmonella paratyphi B) possesses "antigens" 1, 4, 5, 12 of which 4 is peculiar to group B while the others may occur in other groups. Determinant 4 is due to the 3,6-dideoxyhexose abequose. Likewise "antigen" 9 of group D (e.g., Salmonella typhosa) is due to the dideoxyhexose tyvelose as non-reducing end group; antibodies against this determinant cross react with Pasteurella pseudotuberculosis type IV because this organism also produces on its surface a polysaccharide having a tyvelose end group. On the whole the end group sugars contribute most of the group specificity, the "backbone" structure of the O antigens being common to most salmonellae.

The antigenic determinants of human blood-group mucopolysaccharides have been worked out in even greater detail. The A, B and H substances have been most studied. Two trisaccharides have been isolated from A and two from B substances by partial acid hydrolysis. These have the structure:

A 1  α-GalNAc-(1 → 3)-β-Gal-(1 → 3)-GNAc
2  α-GalNAc-(1 → 3)-β-Gal-(1 → 4)-GNAc
B 1  α-Gal  -(1 → 3)-β-Gal-(1 → 3)-GNAc
2  α-Gal  -(1 → 3)-β-Gal-(1 → 4)-GNAc

(Gal = galactose, GalNAc = galactosamine, GNAc = glucosamine)
They possess strong A and B specificity as measured by precipitin inhibition and by haemagglutination inhibition, suggesting that two types of antigenic determinants are present in each. By alkaline degradation in the presence of sodium borohydride a branched oligosaccharide containing a fucose linked \( \alpha 1 \rightarrow 2 \) to the subterminal galactose in the above trisaccharide has been obtained. The largest fragment thus far isolated from A substance shows considerably more A activity than the first A trisaccharide above and has the structure

\[
\begin{align*}
\alpha\text{-Fuc} \\
\downarrow \\
\alpha\text{-GalNAc}\rightarrow (1 \rightarrow 3)\beta\text{-Gal} \rightarrow (1 \rightarrow 4)\text{-GNAc-R}
\end{align*}
\]

in which R is a reduced unsaturated sugar formed by the alkaline degradation. This reduced pentasaccharide may represent one of the A determinants. A similar fragment in which the terminal GalNAc is replaced by Gal would be the B determinant. By enzymatic removal of the terminal GalNAc or Gal the intact A or B substances are converted to substances showing blood group H activity, and two H-active trisaccharides with structures

\[
\begin{align*}
\alpha\text{-Fuc} \\
\downarrow \\
\beta\text{-Gal} \rightarrow (1 \rightarrow 3)\text{-GNAc} \\
\beta\text{-Gal} \rightarrow (1 \rightarrow 4)\text{-GNAc}
\end{align*}
\]

have been isolated.

Protein antigens differ from carbohydrate antigens in almost all respects. Studies with synthetic polypeptides have shown that they may be antigenic and that their specificity can be attributed to peptide sequences.

For naturally occurring, undenatured, globular proteins there is only a little evidence that specificity is determined by sequences of amino acids. Thus, in the tobacco mosaic virus protein, which has 158 amino acids, a tripeptide that is not terminally located (positions 129-131) carries specificity. On the other hand, studies with many proteins of known amino acid constitution and sequence suggest that specificity is due to secondary and tertiary structures, and that small peptides do not cross react with antibodies to the native proteins.

In one instance, even quaternary structure has been implicated in determination of specificity. The consequences are that analogous proteins (e.g., serum albumins or thyroglobulins) of species having an obvious evolutionary relationship may cross react, but otherwise cross reactions are rare and probably occur only when the functional properties of a protein are so exacting as to allow only a limited number of closely similar structures. Eye-lens protein is perhaps an example of this.
Lipids, as antigens, have received much less attention. When purified, their insolubility in physiological media makes them difficult to study serologically. Cardiolipin is the best known purely lipid hapten; the antigenic specificities of the Forssman family have also long been known to be lipid-bound. A large family of sphingoglycolipids has now been shown to have specific haptenic properties and these are antigenic when cell-bound. The indications are that lipids, glycolipids in particular, may prove to be of great importance in determining the antigenic specificity of tissue cells and we should be prepared for the possibility that they may be important also in the immunology of parasites and pathogens generally. Although the specificities of glycolipids are perhaps predominantly due to their carbohydrate content, the totally different physical and chemical properties of these substances justifies their classification with lipids.

Antigens may also be classified according to the context in which they are examined, e.g., species specific, individual specific, (developmental) stage specific, and organ/tissue specific. Species specificity is due to a complex of many different antigens, some occurring in soluble form (serum proteins) and others cell-bound. Tissue specificity, revealed immunologically, is presumably due to the materials characteristic of a tissue by virtue of its specialized functional role, and such specificity often crosses species barriers. Individual specificity results from the complex of blood group polymorphisms, from allotypes and from histocompatibility antigens (cell-bound allotypes). Parasites frequently show highly specific requirements in respect of the host and organ/tissue in which they settle, but whether this is in any way related to their antigenic structure remains to be determined.

It has been suggested that certain pathogens may have preferences for individuals of particular blood groups and that their activity in the past may have left its mark on the present-day distribution of blood groups, but this idea has little factual evidence to support it so far.

It is important also to mention non-antigenic macromolecules. For example, antibodies have not yet been found directed against the acidic carbohydrate polymers of connective tissue (sulfated and containing uronic acids), whose wide distribution in the body has yet to be reflected in any clear definition of their physiological roles. It is of interest that some bacteria are coated with hyaluronic acid which gives them a non-antigenic protective covering.

In some circumstances, substances that are normally antigenic may fail to elicit immunological reactivity. Immunological competence in most animals, and probably in man, is achieved gradually throughout the later stages of foetal and early post-natal development. Bodily constituents with which immunologically competent cells are in contact in sufficient quantity during this period are accepted as "self". So also may foreign material presented before immunological capacity has been attained; thus a chimera may result from transfusion at birth or when a common placenta is shared by non-dizygotic twins.
Presentation of antigen in later life may also induce specific non-reactivity rather than stimulation. The amounts of antigen required to achieve this stage depends on the "strength" of the antigen, as seen by the reacting host, the duration and amount presented, the quantity of immunologically competent lymphoid cells, and many other parameters.

It may be worth pointing out in this regard a possible analogy between successful parasites and neoplastic cells. Tumour-specific antigens are weak. Presumably antigenically strong, potentially malignant cells are readily recognized as foreign and removed by immune mechanisms. Similar factors may possibly explain the observed variability of host-parasite interactions.

**TABLE 1**

**NOMENCLATURE FOR HUMAN IMMUNOGLOBULINS**

<table>
<thead>
<tr>
<th>Notation for immunoglobulin classes</th>
<th>New usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous usage</td>
<td>New usage</td>
</tr>
<tr>
<td>$\gamma$, 7$\gamma$, 6.6$\gamma$, $\gamma_2$, $\gamma_3$</td>
<td>$\gamma$G or IgG</td>
</tr>
<tr>
<td>$\beta_2A$, $\gamma_1A$</td>
<td>$\gamma$A or IgA</td>
</tr>
<tr>
<td>$\gamma_1M$, $\beta_2M$, 19$\gamma$, $\gamma$-macroglobulin</td>
<td>$\gamma$M or IgM</td>
</tr>
<tr>
<td>Type I, I, B</td>
<td>Type K</td>
</tr>
<tr>
<td>Type II, 2, A</td>
<td>Type L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notation for fragments produced by digestion with pepsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present usage</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>A, C, S (I, II)*</td>
</tr>
<tr>
<td>B, F (III)*</td>
</tr>
<tr>
<td>A piece</td>
</tr>
</tbody>
</table>

* The parentheses enclose terms used for fragments in the rabbit.

For 5S fragments obtained by pepsin digestion and having two antibody sites, the notation would be F(ab')$_2$. The univalent pepsin fragments should be designated as Fab'.

<table>
<thead>
<tr>
<th>Notation for polypeptide chains of human immunoglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin class</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>$\gamma$G or IgG</td>
</tr>
<tr>
<td>$\gamma$A or IgA</td>
</tr>
<tr>
<td>$\gamma$M or IgM</td>
</tr>
<tr>
<td>Type K</td>
</tr>
<tr>
<td>Type L</td>
</tr>
</tbody>
</table>

1.2 Heterogeneity of antibodies

The antibody response to a single antigen involves the formation of complex populations of antibody molecules differing in physicochemical and antigenic properties as well as in the configurations of the antibody combining sites, which may be complementary to different determinants on the antigen itself. Although this concept originated almost two decades ago, it is only in the last few years that the importance of heterogeneity has begun to be appreciated and some insight has been developed into its structural basis. Much of the insight has been derived from “experiments of nature”—myelomatoses, the macroglobulinaemias and the Bence-Jones proteins—that revealed an equal complexity among various globulins of serum, and from other “experiments of nature”—the hypogammaglobulinaemias—that resulted in individuals with a defective synthesis of serum globulins and of antibodies as to render them susceptible to many infections. Current interpretations of antibody heterogeneity have merged the two completely different lines of development into the present concept of the immunoglobulins.

1.2.1 The classes of immunoglobulin

The development of immuno-electrophoresis by Grabar and Williams revealed that the classical gamma globulin of Tiselius was a serum globulin with a range of electrophoretic mobilities extending from the alpha-2 region through the beta region into the gamma globulin mobility range. This immunoglobulin is now termed γG or IgG. At the same time two other serum proteins were revealed which were originally called β2A and β2M but are now termed γA and γM or IgA and IgM in accord with the recent WHO Nomenclature Conference.1 (See Table 1.)

Cases of hypogammaglobulinaemia have been found in which all three immunoglobulins were absent and others in which only one or two were absent—the individuals concerned generally showed greatly impaired capacity to form antibody, although two healthy individuals in whom γA is undetectable have been reported. In some chronic infectious diseases, such as malaria and trypanosomiasis, a general rise in total γG or γM immunoglobulin respectively occurs. Cases of myeloma and macroglobulinaemia have been found to involve any of the three types of immunoglobulin. The abnormal globulins in the serum of these patients apparently result from the uncontrolled synthesis of relatively homogeneous proteins representing a small region of the relatively broad electrophoretic mobility range of the normal immunoglobulin. Myeloma proteins and urinary Bence-Jones proteins have been classified into two broad groups by immunochemical

studies. The myeloma globulin and the Bence-Jones protein of a given individual are always of the same group. The basis for this immunological classification is now known to be due to different antigenic determinants K or L on the light chains (see below) of the immunoglobulins. Due to these two types of antigenic determinant, normal serum can be shown to contain at least six immunoglobulins, i.e., γGK, γGL, γAK, γAL, γMK, γML. Antibodies have been demonstrated to occur in each of these six types of immunoglobulin.

The above description applies to human immunoglobulins. Parallel studies in other species (rabbit, mouse, guinea-pig, horse, etc.) have clearly shown the presence of three classes of immunoglobulin and in many instances antibodies have been associated with these proteins. In the mouse and guinea-pig, evidence has been obtained which has been interpreted as indicating the existence of a fourth class of immunoglobulin.

There are important differences in physicochemical and biological properties among the three main classes of immunoglobulin as shown in Table 2. Other properties are considered in section 4.1.3.

<table>
<thead>
<tr>
<th></th>
<th>γG</th>
<th>γA</th>
<th>γM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation constant</td>
<td>7</td>
<td>7-11</td>
<td>19</td>
</tr>
<tr>
<td>Carbohydrate content %</td>
<td>2.6</td>
<td>10.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Passage through placenta</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inactivation by mercaptoethanol</td>
<td>+</td>
<td>+</td>
<td>Ab</td>
</tr>
<tr>
<td>Light chains (molecular wt. 20000)</td>
<td>κ, λ</td>
<td>κ, λ</td>
<td>κ, λ</td>
</tr>
<tr>
<td>Heavy chains (molecular wt. 50000)</td>
<td>γ</td>
<td>α</td>
<td>μ</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Serum Amniotic fluid Bile</td>
<td>Serum Parotid saliva Colostrum Lacrimal fluids</td>
<td></td>
</tr>
</tbody>
</table>

The dynamics of the synthesis of immunoglobulins and of antibodies is of fundamental importance. γM antibody generally appears after initial stimulation by many antigens and in many species. It is followed by the production of γG antibody. γM antibody synthesis may then cease or may go on at a slow rate. In man, irrespective of age, antibody to the O antigens of Salmonella appears to remain mainly of the γM variety. A major question to be settled is whether γM antibodies and γG antibodies are made successively by the same cells, or whether different cells are stimulated independently to make one kind of antibody only. In the horse,
γG antitoxins appear to be formed first. On prolonged immunization an
electrophoretically faster immunoglobulin, which may correspond to γA
globulin, is produced and synthesis of both types of immunoglobulin
continues for long periods.

1.2.2 Structural studies by enzymic digestion

Insight into structural basis for the heterogeneity of the immunoglobulins
has come from degradative studies involving reduction of disulfide bonds
with and without enzymatic digestion. Since the early 1930’s, peptic diges-
tion of horse antitoxins has been known to yield a product that possesses
the full antitoxic activity, but only two-thirds of the molecular weight of
horse γG immunoglobulin. The structural significance of this observation
remained unappreciated until 1958, when Porter showed that digestion of
rabbit antibody or γG immunoglobulin with papain (activated by cysteine)
produced three chromatographically separable fragments. Two of the
fragments now designated Fab possessed intact antibody-combining sites.
By equilibrium dialysis measurement using antibody to a known hapten,
these were subsequently shown to possess the same range of binding affi-
nities as did original intact antibody. The Fab fragments were monovalent,
while the original antibody was bivalent. The third fragment, Fc, was
devoid of antibody activity but contained many of the antigenic determi-
nants of whole γG immunoglobulin. Subsequent studies with antibody γG
immunoglobulin showed that peptic digestion destroyed one third of the γG
immunoglobulin to give a bivalent fragment, F(ab)₂, which is held together
by a disulfide bond, reduction of which splits the fragment into two
monovalent fragments, Fab. Further chromatographic studies revealed that
although two distinct Fab fragments are obtained from most preparations of
γG antibody globulins, this is due to the fact that such preparations contain
a mixture of molecules, which are individually built up either of Fab
fragment of the first chromatographic type plus the Fc fragment, or of two
Fab fragments of the second chromatographic type plus the Fc fragment.

1.2.3 The light and heavy chains

It was shown that treatment with 0.75 M mercaptoethanol followed by
iodoacetamide split γG immunoglobulin into heavy and light polypeptide
chains separable by chromatography on G-75 or G-100 Sephadex (insoluble
dextran). These findings led to the formulation of a structure for γG
immunoglobulin of two light chains and two heavy chains held together
by disulfide bonds.

In further studies, reduction of normal γG immunoglobulin with mercap-
toethanol in 8 M urea, followed by electrophoresis in starch gel in formic acid,
yielded an electrophoretically slow, heavy-chain band and a faster light-
chain band. Both bands were diffuse. By contrast, when myeloma globu-
lin was treated similarly they produced a series of very sharp light-chain
bands. The number and mobility of the light-chain bands varied for
different myeloma proteins, indicating a still further type of heterogeneity.
When purified antibody was obtained from the serum of guinea-pigs and
examined under these conditions, very sharp light-chain bands were
obtained. Their number and position differed from animal to animal and
with different antibodies, indicating a remarkable similarity between mye-
лома globulins and antibodies when judged by heterogeneity of the light-chain
bands in this system.

1.2.4 Heterogeneity of light chains

When the starch-gel electrophoresis was carried out at pH 8.8, the
degree of heterogeneity of light chains was found to be even greater. Under
these circumstances, unselected normal γG immunoglobulins produced ten
light-chain bands. In patterns of myeloma globulins, one or a few of these
bands were found to predominate, indicating that the myeloma globulins
were the product of multiplication of selected clones. The further complexity
of the light-chain bands was established when each could be shown to be
made up of two populations of molecules some with K and some with L
antigenic determinants. Twenty types of γG immunoglobulin molecules are
possible, based on differences in the light chains alone. Further studies are
needed to establish the relationship of various antibodies to the different
light-chain bands.

The two types of γG immunoglobulin, γGK and γGL would thus have
the formulae γ₂κ₂ and γ₂λ₂ in which γ represents the heavy chain and κ and λ
the light chains possessing the K and L determinants. There would be ten
varieties of each of these κ and λ light chains with different mobilities.

Heterogeneity of the heavy polypeptide chains has also been observed
during studies of two individuals, who excreted these in their urine. The
heavy chains were shown to differ immunologically.

1.2.5 Allotypic determinants

A variety of allotypic determinants have been found on the heavy chain of
human γG immunoglobulin, the Gm(a) and Gm(b) specificities. The L
chains contain another set of genetic determinants called the Inv determin-
ants, Inv(a) and Inv(b). There is some evidence that these determinants
are influenced by the quaternary structure of the molecules.

Studies of myeloma globulins from individuals have revealed that a
myeloma globulin possesses only a single kind of Gm determinant and a
single kind of Inv determinant. However, individuals in whom the normal γG
immunoglobulin is Gm(b+) may produce myeloma globulins which are either
Gm(b+) or Gm(b-). Thus myeloma globulins appear to be a result of
neoplastic changes involving a single clone of cells. Of special significance
is the finding that the myeloma globulin has never been reported to possess a Gm determinant lacking in the whole γG immunoglobulin. Similar findings have been observed for the Inv determinants in myeloma.

1.2.6 Antibody production by selected cell populations

Studies of purified antibodies to a variety of antigens have shown that the antibody molecules, just as the myeloma globulin molecules, often represented a selected population with respect to the Gm determinants. Thus an individual whose whole γG immunoglobulin was Gm(a+b+−) produced three antibodies, antidextran, antilevan and antileichoic acid of *S. aureus* which were Gm(a−b−−). A fourth antibody, anti-A was Gm(a+b−−) and a fifth antibody, antitetanus toxoid, was Gm(a+b+−). Findings in other individuals gave the same type of results with respect to Inv determinants.

In man not subjected to repeated antigenic stimulation, it appears on present evidence that antibody production remains confined to clones of the type first stimulated. Thus in a Gma+b+ individual, antidextran antibodies assayed 12 years after immunization were Gm(a−b−−) while the tetanus antitoxin studied shortly after a primary immunization was Gm(a+b+−).

1.2.7 Heterogeneity of the antibody-combining site

None of the manifestations of heterogeneity of γG immunoglobulins described thus far appear to be related to the antibody-combining site itself. During the antibody response to a single antigen, however, substantial heterogeneity may be observed among the antibody-combining sites themselves. A single protein or heteropolysaccharide antigen contains multiple antigenic determinants, and populations of antibody molecules are formed to a number of these determinants. Such immunochemical heterogeneity is readily apparent from the presence of populations of antibodies which cross react with closely related antigens and other populations which fail to cross react in this way.

Will a single antigenic determinant give rise to antibody molecules that are homogeneous with respect to their antibody-combining sites? Evidence obtained from studies on a homopolysaccharide such as dextran indicates that antibody formed even to this antigen is not homogeneous. The combining sites of the populations of antibody molecules produced differed in their binding affinity for oligosaccharides of the isomaltose series. It has been possible to fractionate the antidextran formed by a single individual into two fractions of antibody molecules by adsorbing the antidextran on an insoluble dextran (Sephadex), washing to remove non-antibody protein and then eluting part of the antibody with isomaltose or isomaltotriose and the remaining antibody with isomaltohexose. The two antibody fractions
obtained showed substantial differences in the relative binding affinities of the oligosaccharides to one another, the fraction obtained with the smaller oligosaccharides being relatively readily inhibited by the smaller oligosaccharides. The fraction obtained by subsequent elution with the hexasaccharide is only very poorly inhibited by isomaltose and isomaltotriose. Thus it may be expected that when single antigenic determinants are isolated from complex antigens, their homologous antibodies will still consist of heterogeneous populations.

The complexity of the antibody response is naturally reflected in very different biological properties of individual antisera. This, notably in horse antitoxic sera, has greatly complicated the correlation of the protective potency with antibody content. It would now appear that the protective power of antitoxic sera does not involve all of the antigenic determinants of the toxin molecule and those determinants which give rise to non-protective antibody complicate in vitro assays for antitoxic potency. Antibodies of different classes vary per unit weight in their sensitizing and complement-fixing capacity.

It will become important, as purified antigens of parasites become available, to study the biological properties of the various kinds of antibody formed to these materials. Studies directed towards learning how to achieve maximum production of antibody molecules with a given biological property are sorely needed.

1.3 Cytolytic and cytotoxic effects of antibody

Animals which have made an immunological response to an antigen are sensitized to this antigen, and further contact with the antigen may produce physiological or pathological effects which differ qualitatively and/or quantitatively from the effects produced by the same amount of antigen in an unsensitized animal. Such differences in response were clearly recognized at the beginning of the century by von Pirquet, who coined the term "allergy" to describe them. The reason for these differences is that in the sensitized animal the antigen interacts with pre-existing antibody (resulting in the phenomena of immediate-type hypersensitivity), or with sensitized cells of the lymphoid-macrophage system (leading to the phenomena of delayed-type hypersensitivity), or with both. Recent advances in immunopathology have thrown light on the mechanisms responsible for the various phenomena observed in allergic responses. These may be regarded as by-products of the immune response, which may in certain circumstances have advantages for survival, or whose disadvantages are outweighed by the over-all benefits conferred by the capacity to produce such a response. Nevertheless, in certain circumstances immediate or delayed-type hypersensitivity reactions may be important factors in the pathology of infectious diseases.
1.3.1 Effects of combination of antibodies with antigens which are part of or attached to the cell surface

Combination of antigen with antibody in the presence of complement commonly brings about the "fixation" or activation of complement. This is due to the ordered sequential activation of complement components, some or all of which exist in the tissue fluids in a precursor state, and are converted to enzymatically active forms by the preceding member of the complement series. The initial reaction is triggered in an unknown way by the presence of immunoglobulins aggregated with a suitable spatial orientation, of which antibody-antigen complexes form a special example. The capacity to initiate this sequence of events requires the presence of a specific structural part of the immunoglobulin molecules, and there are marked differences between molecules of one class of immunoglobulin and another in their capacity to activate complement of different species. In the case of the complements of man and the guinea-pig, which have been most extensively studied, the activated forms of the first three components of complement are esterases, but the nature of any enzymic activity elicited at later stages of activation is not known.

When combination of antibody takes place with an antigen which is part of, or closely attached to, a cell membrane, and complement is activated close to the membrane, the end result is the formation of a substance that can damage the membrane of a variety of cells including erythrocytes, lymphocytes, tumour cells, mast cells and Gram-negative bacteria. The damage is manifested by the formation of holes in the membrane, probably in the lipid layer. The effect of such holes is to destroy the osmotic regulation of the cell, and directly or indirectly to cause irreparable damage.

The effectiveness of antibodies of different classes in bringing about complement-dependent lysis of erythrocytes has been found to vary markedly. Whereas one or two IgM antibody molecules per cell are sufficient to activate complement so as to form a hole in the membrane, in the case of IgG antibodies it appears that two molecules must become attached at closely neighbouring sites on the cell, and consequently many more molecules must be attached per cell to produce the same effect. If this observation is also found to apply to lysis of Gram-negative bacteria by antibodies against the O (somatic) antigens, it may provide an explanation, in terms of biological survival value, for the evolutionary persistence of the known capacity of animals to produce a rapid, often transient IgM antibody response, especially to particulate antigens—which precedes the usually greater and more prolonged IgG antibody response. Compared with IgG antibodies, however, IgM antibodies appear to be relatively inefficient at neutralizing toxins or inactivating viruses, and the biological survival value of IgG antibodies is self-evident. It may be that antibodies in each of the different antibody classes will prove to have special biological advantages
under certain conditions. The association of skin-sensitizing antibodies with apparent protection against certain helminth infections may be cited as an illustration of this principle in the field of parasitology.

1.3.2 Effect of combination of antigen with antibody adsorbed to cell surfaces: immediate-type hypersensitivity of the anaphylactic type

Many immunoglobulins are reversibly adsorbed on the surface of tissue cells, more or less firmly. It appears that the antibodies that adsorb firmly on spleen cells, or on macrophages, may be distinct from those that adsorb on the surface of other tissue cells, such as epidermal cells or mast cells. In addition, there are differences between various species of animals in the nature of the antibodies that attach to cells. These differences have not been elucidated except in the case of those antibodies that attach to and sensitize mast cells. The latter may be largely responsible for phenomena of acute anaphylaxis.

Interaction of antigen with suitably adsorbed antibody on the surface of mast cells leads to the rapid disruption of the cells and to the transient liberation of pharmacologically active substances, of which histamine, 5-hydroxytryptamine, SRS-A and bradykinin have been clearly identified. The first two are derived directly from mast cells, though in certain species they may also be liberated from platelets damaged by antigen-antibody reactions of the type involved in immune adherence. These pharmacologically active substances may act on susceptible tissues (vascular epithelium, smooth muscle) either locally or at distant sites, causing extravasation of fluid (but not haemorrhage) and smooth-muscle contraction. The relative amounts of the different pharmacologically active substances liberated, and the main susceptible tissues on which they act, vary from species to species. The result is local or systemic anaphylaxis.

The nature of the antibody responsible varies with the species. In some (including man, monkey and probably the rat) thermolabile IgA immunoglobulin antibody to some antigens has been shown to be the main immunoglobulin responsible, but the correlation is not complete, and further research may show that immunoglobulins of other classes sometimes may produce similar effects. Such IgA immunoglobulins do not cross the placenta, but are selectively concentrated in glandular secretions (e.g., saliva, milk). In other species (e.g., the guinea-pig) antibodies with rapid electrophoretic mobilities have sensitizing activity. The amounts of suitable antibody needed to sensitize tissues are exceedingly small, and may be undetectable by any but the most sensitive techniques. There is evidence that certain antigens, or antigens presented by certain routes (e.g., via the lung), or with certain adjuvants, are especially capable of eliciting production of sensitizing antibody. The factors involved are in urgent need of further investigation.
When antibodies formed in one species are used for passive sensitization of another species, their activity depends upon the two species involved, and it does not follow that the class of antibody that can sensitize the tissues of other members of the donor species will sensitize the recipient species. Furthermore, there is marked heterogeneity in sensitizing ability between antibodies of the same immunoglobulin class from the same individual donor.

Sensitization can be blocked or even reversed by two distinct means:

(a) by the presence of sufficient amounts of non-specific immunoglobulin capable of competing with antibody for the relevant adsorption sites, or

(b) by the presence of sufficient specific antibody, of a class which does not sensitize the tissue, to combine preferentially with the antigen and prevent the latter from reaching the specific sensitizing antibody on the cells.

The nature of the immunoglobulins in relation to their ability to produce these effects requires further study. Serum complement (as defined in haemolytic systems) has not been found necessary for anaphylactic reactions, but most cell disruption involves a series of events which resemble (e.g., in their metal requirements and susceptibility to certain inhibitors) some of those identified as occurring in immune haemolysis.

Although the most obvious effects of antigen combining with antibody absorbed on cells are attributable to mast cell disruption, it is possible that other cells may be damaged in a less obvious way. Since sensitizing and non-sensitizing antibodies usually coexist it may be expected that the pathological changes caused by antigen-antibody complexes (described in section 1.4) will also occur to some degree. Furthermore, anaphylactic reactions are followed shortly by a local or systemic increase in eosinophilic leucocytes. These cells are attracted in an unknown way by some consequence of antigen-antibody interaction, especially when this is of the anaphylactic type, but are not primarily involved. That is, eosinophilia is an indicator of a preceding antigen-antibody interaction.

1.4 Pathology of antigen-antibody complexes

Tissue injury can be produced by circulating antibodies in two general ways. In the first, host antibodies to exogenous or endogenous antigens, immunologically quite unrelated to the tissue injury, may upon combination with the antigen produce a variety of tissue lesions. These injuries vary from transitory but sometimes fatal anaphylactic reactions to acute or subacute necrotizing, inflammatory lesions and slowly developing hyaline degenerative changes. Anaphylactic reactions are discussed in detail in section 1.3.
In the inflammatory, necrotizing and degenerative types of focal tissue injuries produced by reactions of antibody with antigens immunologically unrelated to the tissue injured, the antigen-antibody complex itself appears to act as the etiological agent. The properties of biologically active complexes are as follows:

1. The complexes themselves are toxic.
2. Complexes in moderate antigen excess are most active.
3. Active complexes have affinity for tissues and are usually able to interact with complement.
4. The activity of the complex is dependent upon properties of the antibody, not antigen.
5. The activity of the complex is associated with change in the optical rotation.
6. γG immunoglobulin complexes by chemical linkage in the absence of antigen are aggregated by heat and show properties similar to those of antigen-antibody complexes.

The complex takes on pathogenic properties and localizes in certain tissues inducing focal injury. The protracted nature and low levels of these antigen-antibody reactions make for relatively mild systemic changes. The complexes no doubt exert their injurious effects via humoral mediators, such as complement, and cells, such as the polymorphonuclear (PMN) leukocytes. The temporal and histological characteristics of these various lesions plus their anatomical distribution appear to be largely determined by the logistics of the immunological reaction, i.e., the amounts of reactants available and the duration of their presence. The immunological characteristics of the antigen appear to be of less importance in determining the character of the lesions. Antigen-antibody complexes

1. degranulate mast cells, with liberation of amines;
2. attach to and agglutinate leukocytes and platelets;
3. increase the coagulability of blood;
4. contract smooth muscle;
5. increase capillary permeability;
6. cause endothelial proliferation;
7. attract PMN leukocytes by chemotaxis;
8. are deposited in tissues and produce degenerative change.

The spectrum of lesions induced by antigen-antibody complexes in tissues immunologically unrelated to the reactants extends from acute necrotizing inflammatory reactions to chronic hyaline degeneration. The acute end of the spectrum is exemplified by the Arthus reaction, in which circulating antibody combines with intradermally injected antigen in the vessel walls,
the antigen-antibody complexes react with complement and then attract
PMN leukocytes which, in the act of engulfing and catabolizing the com-
plexes, also cause necrosis of the tissues. Subacute proliferative lesions
develop in classical serum sickness following a single injection of a foreign
protein. In this disease, be it clinical or experimental, soluble antigen-
antibody complexes are present in the circulation for several days during
the host's response to the foreign protein. These circulating complexes
apparently induce and are deposited in proliferative glomerular lesions as
well as lesions of arteries, the myocardium, etc. The chronic end of the spec-
trum is seen when foreign serum proteins are injected daily in small amounts.
Those animals making rather small antibody responses will develop low
levels of soluble complexes in the circulation for weeks or months. These
complexes appear to be deposited along the outer aspect of the glomerular
capillary basement membrane causing a hyaline thickening of the mem-
brane without significant cellular response.

The second general scheme of pathogenesis by which antibodies can
injure tissues involves antibodies directed against antigens in a given tissue,
which, upon reaction with tissue-fixed antigens, cause injury. The demon-
stration of tissue-specific antigens in many, perhaps even all, tissues of the
body provides a theoretical basis for such pathogenic mechanisms. In
addition, antigens in the environment may cross react with some tissue-
specific antigens and thus be able to stimulate the formation of antibody
which therefore may cross react with host tissue antigens. The spontaneous
occurrence or the intentional elicitation of anti-tissue antibodies is frequent,
and their demonstration by in vitro serological techniques is relatively easy.
However, the clear-cut demonstration of tissue injury caused by circulating
autologous or homologous antibodies is extremely difficult and has rarely
been achieved. One possible difficulty in demonstrating the pathogenicity of
circulating anti-tissue antibodies is that the injurious antibodies may be
rapidly bound to the target tissue and never accumulate in the circulation
in amounts detectable by passive transfer. A second difficulty is that the
anti-tissue antibody response is frequently accompanied by a cellular or
delayed-type response to the same or similar antigens and the relative roles
of these two immunological responses in the production of tissue injury are
virtually impossible to separate.

While the demonstration of the pathogenicity of autologous or homo-
logous anti-tissue antibodies is difficult, the mode of action of anti-tissue anti-
bodies has been studied in the experimental model, nephrotoxic serum neph-
ritis. Here a heterologous anti-kidney antibody is used. The antibody reacts
with antigens of the glomeruli of the recipient, inducing immediate damage,
and then remains fixed there. When the host later makes antibody to the
heterologous nephrotoxic gamma globulin, the host antibody combines with
the heterologous immunoglobulin fixed in the glomeruli and further damage
ensues. Although this is not an autologous antigen-antibody system, it
does utilize host glomerular antigen in the first phases of the disease, and host antibody to an exogenous antigen planted in the glomeruli in the second. The injury induced by these reactions between antigen and mammalian antibody is mediated at least in part by serum complement, which provides a chemotactic stimulus for polymorphonuclear leucocytes. Because of the nature of the antigen and/or antibody or perhaps the anatomical localization of the reaction, systemic manifestations are minimal. The anti-kidney antibodies localize in the glomerular capillary wall on the inside of the basement membrane. In order to induce immediate proteinuria, an amount of anti-kidney antibody capable of reacting with half the available antigenic sites of the kidney is needed. This need for large amounts of antibody indicates the ability of the basement membrane to withstand sizeable immunological insults. In this immediate reaction IgM antibodies of the 19S type appear to be 60-100 times more injurious than IgG antibodies of the 75 type. However, if a few molecules of heterologous anti-kidney antibody are fixed in the glomeruli and the host is induced to make antibody to the heterologous immunoglobulin, fixed antigens occupying approximately 1% of the filtration surface will serve as a receptor for a continuing antigen-antibody reaction capable of inducing significant renal damage within weeks or months. These two extremes of injury—acute, demanding exceedingly large amounts of antibody, and chronic, demanding rather small foci of continuing reaction—may set approximate quantitative limits for immunological reactants in the induction of vascular injury by anti-vessel antibodies.

Although it has often been suggested that auto-antibodies to kidney are the cause of human and experimental glomerulonephritis, little evidence for this is available. In nephrotoxic serum nephritis, the possibility that a true auto-antibody against kidney is a significant pathogenic factor seems unlikely. On transplantation of normal isologous kidneys to rats in different stages of nephrotoxic nephritis, these transplanted kidneys remain normal for several months, while the disease in the host's own kidneys progresses, apparently as a result of host antibody reacting with the heterologous gamma globulin in the glomeruli.

Careful immunohistological and electron-microscope studies of the renal lesions produced by antibody via these two different pathogenic schemes have revealed distinctive characteristics of each. The site and form of the antigen-antibody deposition appears to vary with the pathogenic mechanism. In the case of antigen-antibody complexes unrelated to kidney, the complexes are deposited irregularly along the epithelial aspect of the glomerular basement membrane; in the case of anti-kidney antibodies, the antigen-antibody reaction occurs relatively uniformly along the endothelial surface of the basement membrane. Whether distinctive features of immunologically induced lesions in non-renal tissues will similarly indicate the pathogenic mechanisms involved remains to be seen. However, in the case of human nephritis, the immunohistological and ultramicroscopic
features of the disease are already being used as a basis for postulating pathogenic mechanisms and studied as guides in therapy and in the determination of prognosis.

1.5 Delayed-type hypersensitivity

Delayed-type hypersensitivity is characterized by a state of hypersensitivity to the antigen that does not depend upon demonstrable circulating antibody and is not transferable by serum from one animal to another. It owes its name to the fact that the visible effects of antigen injected locally into a sensitized animal are relatively slow in onset and die away more gradually than the effects of immediate-type hypersensitivities. Immediate and delayed-type hypersensitivities commonly, but not always, coexist.

Delayed hypersensitivity can be transferred from one animal to another within a species (but better between isogeneic animals) by living lymphoid cells. The primarily sensitized cells are probably small lymphocytes, but there is evidence that macrophages in a sensitized animal are also altered (probably secondarily) in their reactivity to the specific antigen, and may play the main part in the pathological effects of delayed-type reactions.

The phenomena associated with delayed-type hypersensitivity are as follows:

1. Systemic injection of antigen causes specific lymphopenia, maximal after two to four hours, accompanied by fever, and may cause death.

2. Local injection of antigen into the skin causes a slowly developing erythema, progressing to induration and, in severe reactions, to necrosis. There is little or none of the early increase in capillary permeability, or the intense polymorph invasion seen in immediate-type hypersensitivity. Instead there is a local accumulation of lymphocytes and macrophages. By passive-transfer experiments of labelled lymphoid cells from sensitized donors to unsensitized recipients it has been shown that the great majority of the cells that accumulate at the site of antigen deposition are derived from the recipient.

3. Intraperitoneal injection of antigen causes a transient disappearance of free peritoneal macrophages. These adhere to one another and to the peritoneal lining, but are not killed. A probably related phenomenon is that the in vitro migration of peritoneal macrophages, taken from an animal with delayed-type hypersensitivity, is specifically stimulated in the presence of very low concentrations, and inhibited at higher concentrations, of the antigen. Similar effects are observed when antigen is added to macrophages from an unsensitized animal, mixed with a small proportion of macrophages from a sensitized animal.

4. The macrophages of an animal that has a delayed-type hypersensitivity to a microbial antigen, and in which the specific antigen is being
liberated (i.e., a delayed-type reaction is taking place) have a markedly increased capacity for intracellular destruction not only of the specific microbes but of other antigenically unrelated microbes.

(5) Lymphocytes from animals with delayed-type hypersensitivity to antigens of homologous or heterologous cells are in some circumstances able to destroy such cells in vitro. The mechanism is uncertain.

A useful working hypothesis though unsupported by direct evidence, is that in delayed-type hypersensitivity the small lymphocytes (or a proportion of them) have become "sensitized" and elaborated a specific antibody-like material which is present on their surface and enables them to interact with the antigen so that the cells are altered or destroyed on contact with it. This or some other material can be transferred to macrophages (either before or after the "sensitized" lymphocytes react with the antigen), thereby enabling the macrophages also to react specifically with the antigen and in doing so to become altered in their behaviour (activated).

A state of delayed-type hypersensitivity may precede antibody formation in the primary response, and once circulating antibody is formed delayed-type hypersensitivity is more difficult to demonstrate by skin reactions. In the case of certain antigenic stimuli, however (e.g., tuberculin in the form of tubercle bacilli, chemical sensitizers applied to the skin, tissue homografts, gelatin in depot form), the delayed-type hypersensitivity may persist for long periods and may be the predominant type of hypersensitivity. The conditions that lead to this appear to be such that small amounts of antigen in relatively insoluble form are released slowly to draining lymph nodes, but some specific factors (e.g., the cell wall material in wax D from acid-fast bacilli) may have an additional determining effect.

Development of delayed-type hypersensitivity can be diminished or abolished by prior treatment with the same antigen administered in a form that preferentially stimulates circulating antibody.

Cortisone, hydrocortisone and other corticosteroids have a pronounced inhibitory effect on the manifestations of delayed-type hypersensitivity—perhaps because of a specific effect on lymphocytes, perhaps because of non-specific anti-inflammatory properties (e.g., prevention of lysosomal damage). These drugs have a negligible effect on the reaction of immediate-type hypersensitivity directly mediated by pharmacological agents, and only a moderate effect on damage due to massive neutrophil polymorph invasion.

2. CHARACTERISTICS OF ZOOPARASITIC INFECTIONS

The zooparasites differ in several important respects from this simpler unicellular micro-organisms with which immunology has been traditionally concerned. Parasitic protozoa and metazoa are relatively complex organisms possessing well-developed systems of organs or organelles, some of
which have secretory or excretory functions. In addition, the zooparasites typically undergo complicated cycles of embryological development and differentiation, many of which involve both free-living and parasitic stages, a succession of different host species and organ sites, and/or an alternation of sexual and asexual generations. As a result, the possible permutations and combinations of interaction between protozoan and metazoan parasites and their hosts are almost endless. Even among closely related zooparasites (e.g., the common ascarids of domestic animals) markedly differing patterns of host-parasite relationships are observed. *Necatorias vitulorum* of cattle, for example, normally infects its host only prenatally. *Toxocara canis*, an ascarid of dogs, is a species capable of both prenatal and postnatal infection, while *Ascaris lumbricoides* var. *suum* is apparently able to infect swine only postnatally.

Because of their complexity, which implies complex and specialized physicochemical requirements, it is not surprising that very few metazoan parasites have as yet been successfully cultivated *in vitro* through significant portions of their life cycles. In fact, in no instance (with the possible exception of *Neoplectana glaseri*) has a zooparasitic helminth been propagated *in vitro* through several successive generations and, even among the somewhat simpler protozoa, many parasitic species have still not been cultivated in cell-free media.

### 2.1 Life cycles of zooparasites

The life cycles of parasites such as Plasmodium, Entamoeba, Ascaris, Fasciola and Taenia are generally known among biologists, at least in their basic outlines. Yet even among these few genera of parasites, a startling variety of life patterns is represented. The human *Plasmodium* species, after infecting man, passes through a series of asexual stages which occur successively within the cells of the liver (and possibly in certain other organs), within erythrocytes and transiently free in the plasma. Sexual forms are eventually formed in the human host, but transmission to a new human host takes place through the medium of the mosquito, in which the plasmodia complete an equally complicated sexual cycle, which involves passage through or development within a number of the invertebrate host’s tissues and organs.

*Entamoeba*, on the other hand, may live in the mucosal wall of its host’s intestine (and/or within various other organs), in the lumen of the gut and, outside its host, as a resistant, relatively dormant stage. Similarly, *Ascaris* possesses both free-living and parasitic stages, but during its development in the body of its host it moults through a series of larval stages and passes successively from the gut to the circulatory system, the liver, the lungs, the trachea and finally, via the pharynx, oesophagus and stomach, back again into the intestine. There it matures
within the lumen and passes its resistant eggs into the outside world in the excrement of the host.

Even more complex than the cycles of these parasites is that of helminths of the genus *Fasciola*. As is typical of all digenetic flukes, these parasites of the biliary tree of man and several other mammals infect as free-living larvae certain aquatic snails in which they pass through a complicated succession of both developmental and propagative stages before emerging again as free-living larval cercariae. Finally, tapeworms of the genus *Taenia* require not one but two mammalian hosts to complete their cycle. Following larval development in specific organs of one mammal, they are able to reach maturity only in the intestinal lumen of a second mammalian species, one which utilizes the first host as food.

2.2 Factors influencing infection, development and the course of migration in the host

Recent years have witnessed a considerable advance in knowledge of the physiology, metabolism and biochemistry of these and other zooparasites, but in only a few instances (e.g., *Hymenolepis diminuta, Ascaris lumbricoides*) has sufficient information accumulated about any single host-parasite relationship to cause a discernible picture to emerge from fragments of the research mosaic.

There is, for example, little precise information on the factors governing the eventual localization of zooparasites within their hosts or determining the complex course of migration that many helminths undergo. The present extent of such knowledge is obviously greater for some parasites than for others. Because these intricacies of its life cycle are relatively well understood, the important nematode of sheep, *Haemonchus contortus*, has been chosen to illustrate some of the lines that recent investigations of this type have followed and some of the mechanisms of zooparasitism that have been elucidated.

For infection to occur with *H. contortus*, a nematode which passively enters its host as a free-living larva via the gut, the host must provide a stimulus that acts on the free-living infective larval stage of the worm in such a way as to cause it to commence development as a parasite. Infective larvae of this abomasal parasite receive such a stimulus as they pass through the sheep's rumen.

In *H. contortus*, this stimulation to parasitic life is associated with the process of larval exsheathing. The principal factors that cause exsheathment of *H. contortus* are a temperature of about 38°C and dissolved gaseous carbon dioxide at appropriate concentrations. The action of these factors, especially at relatively low concentrations of carbon dioxide, may be enhanced by reducing agents. The hydrogen-ion concentration, indepen-
dent of its effect on the concentration of dissolved gaseous carbon dioxide and the redox potential, is also important. This stimulus acts as a "physiological trigger". The whole process of exsheathment takes about three hours at 38°C, but if infective larvae are incubated in the stimulating medium for only 15 to 30 minutes, washed, and then incubated in water, the process still goes to completion in about the same period of time.

Other parasitic nematodes that infect the host via the gut require a stimulus similar to that needed by Haemonchus contortus. Trichostrongylus axei, for example, also requires carbon dioxide for exsheathment, but lower concentrations than that needed as a stimulus for Haemonchus contortus are effective. Trichostrongylus colubriformis exsheaths in the abomasum rather than in the rumen, and the stimulation is effected only at high concentrations of hydrogen ions. Moreover, reducing agents have no effect on exsheathment in this latter species.

In both Haemonchus contortus and Trichostrongylus colubriformis these moulting stimuli cause the infective larva to secrete leucine aminopeptidases, which attack the larval sheaths at circumscribed regions so that the contained juveniles are freed. The action of this enzyme, once it is secreted, is, however, independent of the presence of the stimulus. The leucine aminopeptidases of these parasites differ from each other and also from mammalian leucine aminopeptidases in the range of substrates they attack. Thus the enzyme secreted by Haemonchus contortus will not attack the sheaths of Trichostrongylus colubriformis and vice versa. Mammalian leucine aminopeptidase has little effect on sheaths of either species. The enzymes from the parasites do, however, have features in common with the mammalian enzyme. All the enzymes hydrolyse L-leucinamide and L-leucine-β-naphthylamide, require Mg^{2+} or Mn^{2+} as a co-factor, are inhibited by versene (though diisopropyl fluorophosphate is without effect), have their maximum activity about pH 9.5 and are unusually labile.

Similar combinations of physical and chemical factors may operate to "trigger" each of the developmental changes in the life cycles of zooparasites. In addition, sensitive physical and chemical receptors in the parasite may guide it in its often complicated migrations in the body of its host. There is evidence, for example, that Schistosoma mansoni is attracted to a variety of indole derivatives, several of which are present in high concentrations in the mammalian gut and presumably also in the venous plexuses which surround it.

Among the more interesting facets of most zooparasitism is their relatively high degree of host specificity, at least in so far as the ability to complete the life cycle is concerned. Comparatively few data are available in the case of most parasites to explain the phenomena of natural resistance to infection or parasite development that are frequently observed. Among the interesting mechanisms that have thus far been elucidated is the "urea
barrier" in elasmobranchs, which apparently bars their parasitism by helminths other than certain specialized groups of tapeworms that not only tolerate high concentrations of urea but apparently require it for permeability control and osmotic regulation. Another example of such a mechanism is provided by the striking effects of different bile on the larval scolecites of the hydatid tapeworms, *Echinococcus granulosus*. Biles rich in conjugates of desoxycholic acids (which predominate in herbivores) rapidly lyse scolecites, while those rich in cholic acid conjugates (which predominate in carnivore biles) do not.

Similarly interesting experiments have shown that opium-treated mice may readily be infected with *Hymenolepis diminuta*, normally an intestinal parasite of rats. The explanation advanced for the usual inability of this species to infect normal mice is that the infected cysticercoids pass completely through the relatively short digestive tract of the mouse too quickly to respond to "triggering" stimuli that would otherwise enable them to establish themselves. Immunoparasitological studies are necessarily complicated—and at the same time made more interesting—by disclosures of natural resistance mechanisms such as these.

3. SOME CHARACTERISTICS OF IMMUNITY
IN PARASITIC DISEASES

The application of immunological concepts to parasitology is only recent. Even though a variety of immunological phenomena, some of them unique, have been observed in zooparasitisms, comparatively few practical advances have been made in this field. In only a single instance (*Dictyocaulus viviparous* infection in cattle), for example, has a useful immunization technique (irradiated vaccine) against a metazoan parasite been evolved. Similarly, while the classical tools of immunodiagnosis (complement fixation, precipitin and skin reactions) have been applied to a variety of zooparasitisms for the last half century, in no instance in parasitology has the degree of sophistication or success achieved through their use in bacteriology and virology been reached.

This slow advance is due partially to the fact that few immunologists have been sufficiently conversant with the problems of parasitology to seize upon the opportunities for basic immunologic research that exist in this field. Protozoa and metazoa may possibly afford more suitable models for some research subjects than the more familiar bacteria and viruses. Because of their size and the nature of their reactions to the immune responses of the host, the parasitic protozoa and metazoa, for example, provide an unusual opportunity for studies on the actual biochemical effects of antibody on the metabolism of living parasitic organisms. This
relative lack of progress in immunoparasitology is also due, however, to the intricacies of the host-parasite relationships involved.

3.1 Natural resistance

The fact that some zooparasites are much more restricted than others in their range of hosts has already been commented upon. Many different factors may be involved, such as exacting nutritional requirements or very specific conditions needed for triggering development; mechanical considerations related to initial entry and subsequent migration or persistence; or the presence in unsuitable hosts of toxic substances, including natural antibodies. Understanding of why certain parasites cannot dwell successfully in one host might be of great help in explaining why they can do so successfully in another, and thus in controlling them. Some examples are given of limited studies of this aspect of parasitism.

3.1.1 Trichinella spiralis

Very little host specificity is observed in trichinosis. Most mammals can serve as natural or experimental hosts to T. spiralis. Birds can be experimentally infected, although with some difficulty. Poikilothermic animals are usually resistant to infection with this parasite; however, frogs and some other cold-blooded vertebrates have been infected by raising their body temperature. Some other factors that influence the susceptibility of various hosts to this parasite have been studied. Adrenalectomy in mice lowers their natural resistance. On the other hand, addition of milk in the diet, abnormal increases in body weight, water deprivation before or after infection, and intercurrent infection with Ancylostoma caninum appear to increase the natural resistance of experimental animals. Natural resistance is also decreased in mice, guinea-pigs and hamsters by concurrent infection with tuberculosis, by administration of ACTH or cortisone, and by whole-body irradiation. The age of the host also influences resistance to T. spiralis infection. Age resistance has been observed in dogs, as well as in other animals.

3.1.2 Schistosoma spp.

The three principal species of human schistosome differ markedly in their host ranges. In nature, Schistosoma mansoni and S. haematobium are found primarily in man and only occasionally in other mammals, mainly rodents. In contrast, S. japonicum is found naturally in human beings and most types of farm animal, as well as in rodents and other wild species. This comparatively broad host range is observed also among most of the non-human mammalian schistosomes. In the intermediate molluscan host, all of the human schistosomes have a very strict host specificity. At present, our information on natural resistance is based chiefly on infections in
experimental animals. In experiments with *S. mansoni* the number of worms developing in laboratory animals on a deficient diet is usually greater than in animals on a normal diet. Rats on a vitamin-A-free diet are known to have less resistance to experimental infections with *Schistosoma mansoni*. The host's diet may have a marked effect on the schistosomes as well as on the pathological changes produced by them. A diet deficient in cystine, selenium and vitamin E was found to have a profound effect on the course of *S. mansoni* infections in mice.

Clear-cut examples of natural resistance to infection are afforded by trypanosomes. For instance, *Papio* spp. (baboons) are refractory to the inoculation of *T. brucei* sub-group infection, while the fairly closely related *Cercopithecus* spp. are characteristically susceptible. Also, man is susceptible to *T. rhodesiense* but not to the morphologically identical *T. brucei*. The mechanisms underlying these differences are unknown.

### 3.2 Active and passive immunity

Consideration of the natural histories of some of the more serious diseases caused by animal parasites supports the hypothesis that many host populations have survived only because of the development of active immunity to such infections. It is equally evident that such immunity has been purchased by staggering mortality rates, most dramatically illustrated by the extremely high infant mortality rates in many parts of the world in the recent past.

Where the mechanisms operative in natural and acquired immunity against zooparasitic infections have been recognized, they appear to be similar to those operative against infections with bacteria. The emphasis has been on humoral immunity; it is not known whether delayed-type hypersensitivity plays any part. (The analogy that has been drawn between zooparasite invasion and heterografts or homografts cannot be pressed too far, inasmuch as zooparasites do not become vascularized by the host.)

The concept of premunition, developed over many years, envisages a compromise situation in which the host and the parasite both survive and continue to coexist, and supposes that the infectious agent now lives within the host in a latent state. As a host recovers, a state of acquired immunity or increased resistance is attained. A distinction may thus be made between two varieties of acquired immunity. A true sterilizing immunity may develop that continues for a long period following the disappearance of the infectious agent. On the other hand, the immunity that develops may be to superinfection and may persist during a continuing subclinical infection. This non-sterilizing, relative immunity is termed premunition, and exists in some but not all protozoan infections (e.g., piroplasmosis, malaria and coccidiosis), and in many helminth infections.
Although premunition may be a valid entity descriptively, it should be emphasized that it does not stand apart as a separate immunological phenomenon, but can be explained without the necessity of invoking new immunological concepts.

The most important obstacle to a speedy and rational development of immunological studies, including immunization in parasitic infections, is represented by the inability to cultivate zooparasites in defined media in vitro. Consequently, it has frequently been difficult to collect sufficient amounts of parasites or their metabolic products free from host tissues for accurate biochemical studies.

It must be borne in mind that such studies cannot of necessity be safely confined to a particular phase of any parasitic infection, since the nature of the host-parasite interaction depends upon the life history of the parasite and is liable to change at successive stages. Thus in *Haemonchus contortus* infections, the larval stages are primarily responsible for inducing immunity as manifested by the self-cure mechanism in sheep, which involves the elimination of infections in a rather dramatic manner. This reaction can be induced in suitably infected and sensitized sheep by a challenge dose of *H. contortus* larvae. Self-cure is accompanied by a marked rise in circulating antibodies and a significant increase in blood histamine. Although the antibody rise persists, the self-cure reaction may be eliminated by administration of an antihistaminic drug. The abomasal mucosa of the animal undergoing self-cure shows marked oedema and cellular infiltration characteristic of a hypersensitivity reaction. There is some evidence that the molting of *H. contortus* infective larvae from the third to the fourth larval stage is the stimulus for "self-cure".

The mechanisms of resistance to *Ascaris*, which has a relatively long migratory phase (8 days), contrast with those of the rat nematode, *Nippostrongylus*, which, although it also migrates through the lungs, induces its most marked immunity at the adult stage in the bowel. This suggests a relationship between the duration of the tissue phase of a parasite and the stages that induce immunity.

The few parasitic infections discussed below have been chosen to provide examples of the kinds of immunological studies carried out in the parasitic diseases in recent years.

3.2.1 *Trichinosis*

Partial immunity has been produced by repeated intraperitoneal injections, in experimental animals, of living *Trichinella* larvae, heat-killed larvae, or dried and powdered larvae. In all cases, the resistance in the experimentally immunized animals is manifested by the more rapid elimination of adult worms with a resultant reduction of muscle invasion.

In experimental animals, subcutaneous injections of serum from heavily infected rabbits partially protected rats from lethal doses of *Trichinella*
larvae. Serum from infected rabbits passively protected mice and reduced the number of larvae developing from a challenge infection.

3.2.2 Bilharziasis

There is a large body of circumstantial evidence indicating that as a result of infection with schistosomes man develops an acquired immunity to subsequent exposures. The development of acquired immunity to *S. japonicum* in experimental animals following infection has been reported in mice, rabbits and monkeys. Attempts to induce resistance against *S. mansoni* in experimental animals following a primary infection have been only partially successful.

There is some indication that immunity can be acquired in various experimental animals by previous exposure to species of schistosomes that do not naturally infect them, or by exposure to unisexual infections. In some instances, injection of dead parasite material or the media in which parasites have been cultured has also been shown to induce a slight protective response in experimental animals, but living vaccines have always been more effective. Acquired resistance to *Schistosoma mansoni* produced by vaccination with irradiated cercariae will permit mice and monkeys to survive otherwise lethal infections.

The basis of immunity in bilharziasis is still obscure. It is not certain which stage in the life cycle of the parasite is mainly responsible for stimulating resistance, but there is no doubt that some immunity can be produced in the absence of sexually mature worms and eggs. The main immunogenic stimulus appears to be related to their metabolic activity, the resistance being produced by an antigen excreted or secreted by the worms, or alternatively by an unstable antigen present primarily in living schistosomal cells.

3.2.3 Ascariasis

Human beings acquire only a partial immunity to reinfection with *Ascaris lumbricoides*. Most of the evidence for acquired resistance to this parasite is obtained from experimental infections with this and related ascarids in laboratory animals. In such cases, it has been shown that the larvae hatching from embryonated eggs are impeded in their migration through the body and are frequently either eliminated through the intestinal tract or are encapsulated in the tissues. The larvae evoke a polymorphonuclear cell response as they pass through the tissues. This cellular activity in immune animals is considerably greater than in those exposed to a primary infection. The cells surround the migrating larvae of the superimposed infection, check their migratory progress and finally destroy them. Even those ascarids that survive and succeed in completing their cycle in experimental animals frequently produce fewer eggs, are stunted, delayed
in their development and eliminated more readily than in non-immune animals.

Injection of disintegrated eggs, introduction of viable eggs under the skin, injection of saline extracts of eggs or of larval excretions, as well as intravenous inoculations of third-stage larvae from lungs, have been reported to produce partial immunity in guinea-pigs. The demonstration of precipitates about larvae placed in immune serum is further evidence of circulating antibodies.

The different developmental stages of ascarids vary in their ability to release antigens that stimulate "protective" antibodies and in their susceptibility to the host response. In *Ascaris* infection it appears that the major part of the immune response is induced by the migratory stages. There is evidence that antigens that elicit protective antibodies are probably released at the moulting period between the second and third larval stages. Larval stages prior to this moulting are apparently poorly antigenic. In *Ascaris* the bowel phase of the infection is also responsible for stimulating an immune response, in particular at the moulting between the fourth and fifth larval stages. This phase of the infection may be associated with a marked loss of the worm burden and in natural infections may provide a regulating mechanism. Excretory and secretory antigens, obtained by *in vitro* cultivation of third-stage *Ascaris* larvae, induce in guinea-pigs a significant protection against a challenge dose. (The guinea-pig, however, is not a natural host for *Ascaris*, which fails to reach maturity in this host.) These antigens are very labile and are destroyed by freezing.

Immune adherence of red blood cells occurs on the cuticle of *Ascaris* larvae in the presence of guinea-pig complement. White cells also become firmly adherent to the cuticle in the presence of antibody, but this reaction is independent of complement. It also seems probable that antibodies behave as opsonins and act in conjunction with the defensive body cells.

### 3.2.4 Metastrongylosis

The metastrongyloid nematode parasite of cattle, *Dictyocaulus viviparus*, has been extensively studied, and it is clear that immunity can be produced in different ways. Immunity is produced under natural and experimental conditions following a severe attack of the disease. By using the serum of a hyperimmunized bovine that has been subjected to repeated challenge, protection can be transferred to susceptible animals. The protection thus obtained is high and, in addition to preventing the clinical disease, prevents the development of 98% of the worms when the host receives a heavy challenge.

A high level of immunity to this parasite is also produced by the use of a vaccine consisting of X-irradiated infective third-stage larvae. While a substantial immunity is produced by a single dose of 4000 irradiated
larvae, clinical effects are nevertheless apparent. The maximum immune response has been produced by using two doses of 1000 larvae given at an interval of a month. Two weeks after the second dose, immunity is at a high level and in some experiments absolute.

There is very little information on the persistence of immunity when animals are protected from challenge. One experiment suggests that the very strong immunity developed to *Dictyocaulus* is fairly low after the first year. On the other hand, animals that have recovered from a severe attack of *Dictyocaulus* infection and are continually exposed to re-infection retain their immunity for life.

3.2.5 *Ancylostomiasis*

Congenital infection with *Ancylostoma caninum* occurs in pups of infected bitches. This infection in endemic areas can cause 50% mortality from anaemia in early life. Pups that survive to adulthood retain only minimal intestinal infections. Females among them may nevertheless retain undeveloped larvae in their tissues that infect the foetus during pregnancy.

Following a single infection with 1000 X-irradiated *A. caninum* larvae, a significant level of immunity can be demonstrated by worm counts following challenge with unirradiated larvae.

3.2.6 *Malaria*

For many years it has been recognized that a strong immunity to malaria is acquired by populations exposed to frequent infection. Further, observations made on therapeutically induced infections have indicated that acquired immunity is species-specific and may also be strain-specific. Recently, field studies, culminating in the successful passive transfer of immunity in man, have demonstrated the existence in the blood of immune persons of a humoral factor or factors capable of dramatically reducing parasitaemia. This factor or factors are in the yG immunoglobulin (section 1.2) and can be transmitted transplacentally from immune mother to offspring. However, the protective antibody probably accounts for only a small part of the marked increase in gamma globulin that has been shown to occur after repeated malarial infection.

There is evidence to indicate that naturally acquired immunity in man is effective against only the asexual erythrocytic cycle of development and that it has no detectable effect upon sporozoites, the exo-erythrocytic cycle or the sexual erythrocytic forms. However, this should not be construed as indicating that these forms are necessarily less antigenic, for the sporozoites of avian malaria have been shown to be distinctly antigenic. The essential difference may be quantitative, for it is in the asexual erythrocytic cycle that the parasite in a non-immune person achieves its greatest density intracellularly and extracellularly.
The study of infections treated by immune serum has indicated that effective antibody-antigen interaction may well occur late in the asexual erythrocytic cycle. Whether the accessibility of the antigen is governed by increased permeability of the erythrocytic membrane about the growing erythrocyte-parasite complex or by emergence of the parasite from the red cell is not yet known. In this connexion, it should be borne in mind that, throughout the life cycle of the plasmodium, brief periods of extracellular activity alternate with relatively long periods of intracellular activity.

If this concept of immune mechanism is correct, it follows that, even in a highly immune individual, viable sporozoite inoculation will lead to the successful establishment of normal pre-erythrocytic schizogony and to the normal occurrence of first-generation asexual erythrocytic parasitaemia. This has been shown to be the case in simian malaria. Further, in the immune individual, those sexual forms that originate from first-generation asexual erythrocytic parasites or from pre-erythrocytic precursors may also develop unimpeded. However, since the infectivity of malarious blood for mosquitoes varies in relation to other factors as well as its density of gametocytes, some quite independent immune mechanisms affecting gamocyte “maturity” may be involved. Only at the end of the asexual erythrocytic cycle can acquired immunity act effectively. The implication is, therefore, that, whereas malarial immunity may be similar to that evoked in bacterial infections, a fundamental difference may be that the malarial parasite, after being introduced by the mosquito, is given a prolonged period in which to build up its invading strength before immunity operates.

The outcome of any malarial infection may be expressed as the result of a competition between parasitic multiplication and the ability of the host to limit it. Thus two factors unrelated to the host are important:

1. the quantum of viable sporozoites inoculated;
2. the reproductive potential of the infecting species of plasmodium.

In human malaria, the reproductive capacity of the four infecting species varies greatly. *P. falciparum* can multiply 40,000-fold in the pre-erythrocytic schizont, *P. ovale* 15,000-fold, *P. vivax* 10,000-fold and *P. malariae* 2000-fold. Once the asexual erythrocytic stage is reached, each *P. falciparum* parasite may reproduce itself 16 times in each 48-hour period, *P. vivax* does likewise, whereas *P. ovale* reproduces itself 8 times in 48 hours and *P. malariae* 8 times in 72 hours. Thus it will be appreciated that, judged by its capacity for multiplication, *P. falciparum* is by far the most important of the human species of plasmodia as regards potential pathogenicity. This accords well with clinical experience. When considering the pathogenic potential of any human plasmodial species, it should also be remembered that all parasitic multiplication prior to the establishment of asexual erythrocytic reproduction probably takes place uninfluenced by the immune state of the host. Thus the importance of the magnitude of sporozoite infection...
will be realized. While information concerning the minimum infective dose of sporozoites is lacking, it has been demonstrated that in some species light infection may be followed by a latent period lasting several months.

A further basic factor is whether the infecting species does or does not possess a secondary exo-erythrocytic cycle capable of continuing in the liver despite the presence in the blood of high titres of antibodies effective against asexual erythrocytic forms. The existence of such a cycle in relation to the waxing and waning of humoral immunity specific to asexual blood forms is probably of great importance in the understanding of malarial relapses.

At present the view is widely held that intraspecies variation in antigenicity is both widespread and frequent in the four human species of plasmodia. Experimental studies on which conclusions on intraspecies variation have been based have usually involved the immunization of a subject by infection and subsequent challenge with the same species. Breakdown of immunity to such a challenge has been interpreted as indicating strain variation in parasite antigenicity. When these investigations were made, knowledge of the reproductive potential of the pre-erythrocytic cycle was non-existent, and challenging doses of sporozoites were not carefully controlled. It is possible, therefore, that the breakdown of acquired immunity was more the result of challenging with overwhelming doses than of challenging with an antigenically distinct strain.

Recently West African immune serum was found to be therapeutically effective against East African strains of *P. falciparum*; thus there was no indication of any marked antigenic differences between strains of this parasite in two widely separated regions.

The whole question of antigenic variation in strains of different species should be regarded as open, pending fresh and adequately controlled experimentation. In this connexion, it must be stressed that in areas where infection is exceedingly frequent the parasitic burden of an individual at any given time is likely to have arisen from not one but multiple infections. In attempts to secure presumed antigenically homogeneous populations of parasites this should be borne in mind.

For many years it has been known that in trypanosome infection the protozoon complicates the immune reaction of the host by successively changing its antigenicity. Whether a similar state of affairs occurs in malaria must be considered. It is certainly true that following effective parasiticidal crises in the human host parasites may persist in very low density for some time, and it has been suggested that these survivors may persist because they have changed their antigenicity. Although this may indeed be the case a similar state could arise in other ways provided the malarial parasite is susceptible to immune influences only in the late stages of the asexual erythrocytic cycle. For example, low densities of asexual blood forms could be maintained in an immune subject by spillover of successive broods from persisting primary or secondary exo-erythrocytic
forms. Again, any factor, such as a reduction in metabolic activity, that slows the rate of growth of the intra-erythrocytic parasite would delay antigen-antibody interaction and correspondingly prolong parasitic circulation in the blood.

The newborn in hyperendemic circumstances is well known to be refractory to malarial infection for a variable period after birth. This period of relative insusceptibility, ascribable mainly to passive immunity, is inherited from the mother, and it has been demonstrated that γG globulin prepared from umbilical cord blood of Nigerian infants is capable of resolving fulminating parasitaemia in infected children.

Primary malarial infection in young African children tends to be mild and often asymptomatic, but later parasitaemia becomes dense and severe clinical illness is the rule rather than the exception. This period of acute malaria is evident from about six months of age until about two years. Thereafter, clinical illness gradually lessens, although parasitaemia persists in fairly high density. Thus the first real manifestation of acquired immunity, epidemiologically speaking, is the ability to modify clinical illness without marked resistance to parasitaemia. This phase of clinical improvement has long been recognized and has been termed the “antitoxic” stage, although no evidence of any malarial toxin has to date been gathered. Antibodies to substances, e.g., metabolites, formed within the parasite-cell complex and liberated on rupture of the complex, may conceivably be involved in bringing about this improvement.

From this point onwards, parasite densities slowly decline until in adult life a state of balance is reached. Adults seldom present a parasite rate of more than 25% and parasite density is always extremely low, except possibly in pregnancy where some disturbance of the antiparasitic mechanism appears to occur. In this adult phase true antiplasmodial immunity is apparent.

Until recently, no technique has existed capable of measuring immune response to plasmodial infection with specificity. Recently the application of immunofluorescence has given encouraging results. Population studies made in a hyperendemic area of the Gambia have indicated that serum concentrations of fluorescent antibody vary with age in a manner compatible with classical concepts of malarial immunity. Moreover, as rising serum concentrations of this antibody are matched by decreasing density of parasitaemia, the indication is that the antibodies measured by immunofluorescence include at least some related to antiplasmodial rather than to “antitoxic” immunity.

The destruction of red cells in malaria inevitably results in some degree of anaemia in heavy parasitic infections. However, in some instances the degree of anaemia produced appears disproportionately great when the total density of parasitaemia is considered. Some part of this discrepancy may be explained by indiscriminate erythrocytic phagocytosis by the spleen. This discrepancy still persists in splenectomized experimental animals and
therefore the existence of a haemolytic auto-antibody has been postulated. To date no such antibody has been convincingly demonstrated, and the hypothesis remains unproved.

3.2.7 Trypanosomiasis

Wild ungulates and certain breeds of cattle can exist under continuous heavy attack by the vector Glossina without clinical evidence of trypanosomiasis. Their immunity, however, is not a sterile one (i.e., trypanosomes are not eliminated) but simply a state of balance in the host-parasite relationship. Host populations of antelope species, for example, show considerable parasitaemic rates, sometimes over 30%. The information from the field concerning cattle is inconclusive and confusing, probably because of the multiplicity of trypanosome antigenic types to which the animals are exposed. It is not clear whether any existing immunity is absolute or is likely to be overcome by increased challenge.

In the laboratory, animals may be immunized against a given antigenic type of trypanosome either by the inoculation of living organisms or by the injection of serum containing released antigens. Such immunized animals are protected only against the homologous antigenic type (see section 5.3).

In man, but not typically in other animals, the long-established blood infection is followed by invasion of the central nervous system, accompanied by elevation of cerebrospinal fluid protein levels and cell counts. Infections with T. brucei subgroup trypanosomes appear characteristically to induce extreme elevations of the yM immunoglobulins in the serum and, in man at least, also in the cerebrospinal fluid.

In both man and cattle, death from trypanosomiasis is associated usually not with fulminant parasitaemia but with a cachectic condition in which parasites are scanty.

3.2.8 Coccidiosis

In coccidiosis of chickens, it is well established that under natural conditions acquired immunity develops to Eimeria tenella and other Eimeria species but the level of immunity developed does not lead to complete exclusion of the parasite from the host. Nevertheless, it has been shown to be sufficient to protect the host from death, blood loss and the poor growth rate associated with primary infections with this parasite.

An even better level of immunity has been developed in chickens by the use of oocysts attenuated by X-irradiation. By vaccinating chickens at three weeks of age and again at five weeks, deaths and blood loss are prevented, and chickens continue to grow at a normal rate when submitted to an otherwise lethal challenge of half a million oocysts. The vaccine itself sets up a minimal infection in the host, detectable only by faecal examination and not by blood loss.
3.2.9 Leishmaniasis

Infection in cutaneous leishmaniasis (*Leishmania tropica*) is characterized by the formation of a nodule in which marked proliferation of the parasite occurs. The resultant sore eventually heals, following marked local proliferation not only of histiocytes but also of lymphocytes and plasma cells. Such proliferation sometimes occurs also in the draining lymph nodes. After healing there is a solid immunity to reinfection, but if the chancre is excised early no such immunity results. Although antibodies against the leptonad form can subsequently be demonstrated, there is no clear evidence that the immunity is due to antibody. There is evidence of cross protection between *L. tropica* and *L. mexicana* but no cross protection between these forms and *L. donovani*.

In *L. tropica* infections the possible importance with regard to protection of delayed-type hypersensitivity (section 4.1.5) and the enhanced activity of macrophages remain to be evaluated. However, the fact that a solid immunity to this form can be produced by a relatively benign infection suggests the possibility that modification of other strains might produce effective agents for vaccination against the more virulent forms.

In the case of *L. donovani*, the organism spreads to and proliferates within the macrophages throughout the reticulo-endothelial system. Marked hyperplasia of the reticulum cells and macrophages and also of cells of the lymphocyte-plasma-cell series occurs, although parasites are not found in these latter cells. There is a marked concomitant increase in the levels of immunoglobulins in the blood, but no evidence of immunity to the leishmaniasis itself, although antibodies to the leptonad forms are detectable. It has been claimed that persons infected with *L. donovani* are unusually resistant to infection with malaria. Adrenal destruction is not uncommon in visceral leishmaniasis. The mechanism of this destruction is not clear, and it is not known whether the symptoms of Addison's disease are present.

In the case of visceral leishmaniasis the mechanism by which the immunoglobulin increase is caused is a matter of great interest. For example, it is not known whether the immunoglobulins result from a random stimulation of the total immunological competence of the cells, and whether the response to specific antigens is increased or decreased. In other conditions where immunoglobulin increases are consistently observed (e.g., hyperendemic malaria), demonstrable specific malarial antibody appears to account for only a small proportion of the total increase.

3.3 Immune mechanisms in invertebrates

Many zooparasites infect invertebrates during portions of their life cycles. The ability of phagocytic cells of invertebrates to discriminate between foreign matter and normal body constituents has been documented
for many invertebrate groups. These responses to foreign material apparently differ from those of vertebrates, since the invertebrates do not have organized lymphoid tissues, plasma cells or immunoglobulins, which are the essential elements in the vertebrates. Protozoa, such as various species of amoebae, may selectively ingest a particular species of flagellate from a mixed population and can discriminate among nutritive and non-nutritive materials. A chemotactic response may be involved in this type of recognition. Motile bacterial cells may also be concentrated on the surface of an amoeba by a type of agglutination.

In the metazoan invertebrates, cells exist in the body cavity that are amoeboid in character and may occur in a variety of forms. They have been referred to by various terms e.g., haemocytes, amoebocytes, lymphocytes, etc. Among the arthropods, annelids and molluscs, these cells will phagocytize a wide variety of foreign particles of inert or living matter when these enter through natural circumstances, or are deliberately introduced into the body. For example, particles of Indian ink, carmine and living bacteria may be eliminated in this manner. The response to larger particles is somewhat different. The responding cells accumulate on the surface of the foreign body in layer upon layer to form an encapsulating nodule in which fibrils may be identified. Such a response may occur in a matter of hours. Among those invertebrates that act as hosts for various zooparasites, it is evident that the response to an organism that may be considered a “normal” parasite for the host is quite different from that which results when an aberrant parasite enters. Little or no recognition of the natural parasite may occur in terms of a cellular response, and the parasite may develop through its normal cycle in the host, as occurs in the larval stages of trematodes in molluscs and in filarial development in arthropods. On the other hand, schistosome miracidia entering an unsuitable snail will be rapidly enveloped in a nodule, and microfilariae in the haemocoel of an abnormal insect host will be killed by being enclosed within a hard capsular sheath of melanin-like material resulting from the cellular response.

The report of antibody production by invertebrates is controversial, and until now unconvincing. Data have been presented indicating that when bacteria are introduced one or more times into an insect a rise in the number of haemocytes occurs, and that there is a concomitant appearance and subsequent rise in soluble substance, inhibitory or toxic to the bacterial cells. A study on infection of the snail Australorbis glabratus infected with miracidia of Schistosoma mansoni has indicated that immobilizing substance for miracidia can be demonstrated in snail extracts as early as 9 days after infection. Absorption of up to two-thirds of the immobilizing activity of the extracts can be accomplished by exposure to either living or formalin-fixed cercariae. It may not be justified to conclude from evidence of this type that there is differential production of specific reactive substances, nor
that there is a development of a specific hypersensitivity of the tissues to the infecting organism or its products. It is apparent that this area requires further study.

4. HOST RESPONSES IMPLICATED IN IMMUNITY

In general, the effect of host immune response on metazoan parasites is manifested by changes in their numbers, rate or extent of growth, morphogenesis, extent of migration and reproduction rate.

4.1 Specific effects on parasites

4.1.1 Reduction in numbers

Since metazoan parasites are large enough to be readily counted, the reduction of the number of organisms originating from a challenge infection, as compared with controls, provides an index of the degree of immunity. A single measurement can be made at the time of maturity of the parasite, but a more sensitive index is the sequential change in numbers as the life cycle progresses. Thus in several nematode infections it can be demonstrated that the parasite population in an immune animal falls markedly at the time of molting from one stage to another. The mechanisms leading to this effect are probably a combination of the following phenomena.

4.1.2 Inhibition of growth and arrested development

Inhibition of growth is a widespread phenomenon in immunity to parasitic helminths. It may be an over-all effect on the whole population or may affect only a proportion of the population; this latter finding may be due to heterogeneity of the population. In many cases the inhibition of growth (stunting) is manifest at specific morphological or physiological stages in the life cycle, e.g., the molting period in some nematodes.

Arrested development is known to occur in many helminth infections, and is of quite common occurrence. Many types of arrested development are undoubtedly due to the expression of an immune response.

In the case of *Nippostrongylus brasiliensis*, inhibited worms, when taken from an immunized host and transplanted into a non-immunized animal, will go on rapidly to complete development. This indicates that not only are immune processes responsible for the retardation, but also that their constant presence is required if inhibition is to be maintained. In some cases, the retarded growth appears to be associated with the presence of adult worms. Thus, in the case of *Trichonema* spp. of horses, the elimination of adult worms by therapy will allow groups of dormant larvae to emerge from the bowel mucosa and become mature. This process can be
repeated several times in the same host. If an immunological mechanism is involved, it can be envisaged that a lowering of the immune status of the animal will allow larvae to recommence development, but the part played by adult worms in maintaining the immune status above levels that will allow development is unknown. Similarly, with *Haemonchus placei*, a parasite of the abomasum of cattle, large numbers of larvae may remain dormant in the mucosa. On elimination of the adults, larval development is initiated, and severe and even fatal infections may develop.

4.1.3 Inhibition of morphogenesis and reproduction

It is frequently possible to identify the stage of the cycle that is primarily responsible for (and in turn affected by) the stimulation of the immune response by detailed observations of the development stages. This is exemplified by the partial inhibition of development of oogenic tissues in the dog tapeworm *Echinococcus granulosus* by the immune host, and by a widespread comparable phenomenon in certain nematodes in which egg production is found to be specifically inhibited in an otherwise apparently normal parasite. This effect is frequently a most sensitive index of the onset of immunity. The interference with morphogenesis or reproduction may be sufficient to affect essential physiological processes of the parasite and may be manifested in nematodes as an inability to undergo metamorphosis to the subsequent developmental stages.

As with the other phenomena, no detailed knowledge is available on the mechanism of these effects, but the possibility that they are mediated through interference with the function of chemoreceptors is worthy of investigation.

4.1.4 Inhibition of migration

This is to some extent a function of growth and development. It may be manifested by a slowing or a cessation of the normal migratory pattern. It is illustrated well in the immune response to *Ascaris* infection in guinea-pigs, an abnormal host, in which larvae are held up in the liver; in highly immune animals the migration to the lungs is completely suppressed. Such forms whose migration is delayed become susceptible to cellular attack which may further delay their migration or lead to their destruction and removal. Nevertheless, cellular response is not evident in all cases, and the delay in migration may merely be an expression of inhibition of morphogenesis.

In addition to a delay in normal migration, the final site of parasitism may be altered. In immune animals, schistosomes, for example, may move to a more proximal position and may be found in the intrahepatic circulation.
4.1.5 Other effects

More specific effects in physiological systems can be demonstrated in vitro. These include the inhibition by immune serum of enzymes such as lipases and proteases from the oesophageal glands of the dog hookworm Ancylostoma caninum and the inhibition of oxygen consumption of the infective stage of Nippostrongylus muris. Such studies lend support to the frequently held view that enzymes are important as antigens, but although a large number of such antigens must exist in parasitic organisms there is scanty information on the antibodies that may be formed against them. It is of interest to note that although the lactic dehydrogenase of schistosomes differs immunologically from host lactic dehydrogenase, no antibody to the parasitic enzyme has as yet been demonstrated in an infected host. The possible usefulness of immunizing with purified parasitic enzymes is worthy of further exploration.

Information is accumulating on the antigens of specific organs and structures of parasites. Such studies have used both whole organisms and isolated tissues. Thus antigenicity of the cuticle of certain nematodes is well documented. In some instance a cross reaction with blood group A antigens has been observed. The intestinal tract of some nematodes is also known to be antigenic, as observed by the fluorescent antibody technique. It will be important to learn whether or not such antigens of parasite tissues in more or less continuous contact with the host are involved in immunity.

The specific effects on immunity of different developmental stages are well illustrated in the schistosome life cycle. It is possible to demonstrate a variety of immune phenomena against the various developmental stages, ranging from immobilization of miracidia to the formation of precipitates around eggs. While all these reactions can be induced by a single infection, some may be strictly stage-specific, and in no case is there any clear relationship with protective immunity.

4.2 Hypersensitivity

4.2.1 Allergic responses

Many parasitic diseases are associated with hypersensitivity and allergic reactions. Infections with Ascaris lumbricoides in children are characterized clinically by attacks of urticaria, rash, asthma, and other allergic manifestations. Chronic filariasis due to lifelong infection with Wuchereria bancrofti may lead to elephantiasis in a small percentage of infected individuals. The extensive tissue damage and blockage of the lymphatic system may be exacerbated by allergic factors. Patients with filarial disease exhibit a heightened sensitivity to the presence of filarial protein. The administration of small doses of diethylcarbamazine will elicit urticaria and a rash in
individuals infected with filarial parasites. This may be used as a diagnostic tool in endemic areas.

Anaphylactic reactions can be readily induced in animals infected with helminth parasites by the injection of homologous antigen into the bloodstream. For example, guinea-pigs infected with *Ascaris* are very susceptible to anaphylactic shock. Inoculation of extracts of *Trichinella spiralis* into sensitized rats leads to mast cell disruption in the subcutaneous tissues.

Many subjects with helminth infections respond to intracutaneous injection of soluble antigen by developing a weal and flare of the immediate hypersensitivity type. Among protozoan infections (e.g., *Toxoplasma* and *Leishmania*) the immediate skin response is not observed, but delayed tuberculin-type reactions develop in 24-48 hours.

Skin-sensitizing (Prausnitz-Küstner, reagin-type) antibodies have been found in the serum of patients with many helminthiases (hydatid diseases, bilharziasis, filariasis, onchoerciasis, trichinosis, fascioliasis and others). The role that these antibodies may play in the immune response of the host has only recently been investigated. Studies with *Schistosoma mansoni* in the rat indicate that reagin-like antibodies infiltrated into the skin inhibit the penetration of cercariae through the infiltrated area of skin. Experimental models with *Nippostrongylus brasiliensis* indicate that rats infected with this parasite also develop skin-sensitizing antibodies of the reagin type concomitantly with the development of immunity. Other antibodies against the same antigen, but that do not sensitize the skin, also occur but are not correlated with immunity. Some degree of passive protection can be transferred by serum containing skin-sensitizing antibody. These skin-sensitizing antibodies to parasites in animals can be demonstrated only by passive transfer of antibody within the species, and differ from the classical precipitating antibodies, which produce passive cutaneous anaphylaxis when tested in guinea-pig skin (see section 1.3).

### 4.2.2 Eosinophilia

The behaviour of eosinophils is strongly influenced by immunological reactions and therefore, as might be expected, by many host-parasite interactions. The simplest demonstration of this influence is the observation of eosinophil response to combinations of antibody with non-toxic, inanimate antigens. Intraperitoneal injection of an antigen into an immunized mouse results in the formation of antigen-antibody complex and an influx of eosinophils into the peritoneal cavity. Not only do the eosinophils appear to be attracted to immune complexes, but they can be observed to phagocytose them. The complexes alone may not provide the attraction, but may rather mediate it by humoral by-products. This is suggested by the local eosinophilia induced by injection into a normal homologous recipient of minced lung from a guinea-pig suffering from anaphylaxis.
REPORT OF A WHO EXPERT COMMITTEE 45

These experimental situations provide relatively uncomplicated conditions for demonstrating the immunological induction of eosinophilia. Far more complicated are clinical disorders characterized by local and/or systemic eosinophilia. Perhaps the simplest of these are the frank atopic diseases such as seasonal allergy and asthma, and the hypersensitivities such as serum sickness, in which local and sometimes systemic eosinophilia is common. In these cases one can expect the formation of antigen-antibody complexes initiating the condition. Less clear are the poorly understood connective tissue diseases such as polyarteritis nodosa, which may be characterized by local and systemic eosinophilia.

Eosinophilia is a hallmark of most parasitic diseases. The presence of antigen-antibody complexes in parasitic infections has not been intensively studied and the mechanism for induction of eosinophilia in parasitic diseases is not well understood. Sera from patients with relatively high degrees of eosinophilia (20 % or greater) may show antibody titres to a host of parasitic antigens (filariae, hookworms, *Ascaris*, *Fasciola* and *Trichinella*). The clinical syndrome of eosinophilic lung or tropical eosinophilia, which is characterized by infiltration in the lung, a dry cough and a high eosinophilia in the blood, has been described in a large number of patients in Singapore. This clinical entity responds dramatically to therapy with diethylcarbamazine. Within one week after the initiation of treatment the eosinophilia returns to normal values. Since diethylcarbamazine is specific for filarial infections, the etiology of eosinophilic lung was believed to be filarial in nature. Non-human microfilariae of the genus *Brugia* that normally infect cats and monkeys in Malaya were suspected as the etiological agent in man. Recent studies, however, indicate that other non-human helminth larvae (*Ascaris* and *Toxocara*) may also be incriminated. Eosinophilic lung may therefore possibly be a manifestation of visceral larva migrans in man.

The cellular responses to the lungworm *Dictyocaulus viviparus* in cattle have been worked out in some detail, and are of considerable interest because the clinical manifestations of the disease syndrome are due to the host tissue response to the parasite. The larvae quickly penetrate the intestinal wall and produce very little demonstrable reaction, but during passage through the mesenteric lymph nodes a marked giant-cell reaction occurs without phagocytosis of the larvae. When the larvae reach the lungs they enter the smaller branches of the bronchial tree; marked eosinophil infiltration occurs around each larva. In non-immune animals no phagocytosis of the larvae is observed. On the other hand, in immune animals and in animals treated with diethylcarbamazine (which kills the larvae) phagocytosis by the eosinophils occurs. The larvae disintegrate and a small eosinophilic granuloma is formed, to be replaced by a small, highly organized lymph-node-like structure. It is not known whether such structures produce antibody.

As the parasite nears the adult stage, near the end of the third week, the
eosinophil response is replaced by hyperplasia of the bronchial epithelium and greatly increased epithelialization of the alveolar walls. This reaction becomes more intense as the adult fully develops and eggs are produced. The result is a dyspnoeic syndrome.

In severe cases egg output drops sharply after 2-3 weeks of patency and most of the adult worms are swept up the trachea and the dyspnoea gradually subsides. When an adult worm is trapped in a small bronchus and cannot be ejected by the bronchial cilia, a massive necrotic reaction takes place around the disintegrating worm. These lesions develop into large, granulomatous masses which induce more dyspnoea. A similar lesion will develop in a partially immune animal being reinjected, and also following the death of adult worms when treated with diethylcarbamazine.

At the time of elimination of the adult worms from the host there is often a marked worsening of the clinical condition, with death of approximately 25% of the animals. Death is due to a very intense and widespread alveolar epithelialization.

4.2.3 Immunopathology

Immunopathology, the discipline concerned with the pathogenesis of diseases caused by immunological or hypersensitivity reactions, appears to be potentially of great importance in the field of parasitology. The importance of immunopathological processes in viral and bacterial infections of man have long been appreciated; however, this aspect of the parasitic diseases has been investigated to only a limited extent. Parasitism of the mammalian host by antigenically complex organisms results in many antibody responses, some of which may contribute to host immunity. In addition, however, these antibodies are potentially capable of reacting with products of the parasite, or perhaps cross reacting with host antigens, thereby producing immunopathological changes. Such changes would be unrelated to any primary toxicity or pathogenicity of the parasite but rather dependent upon the host antibody response.

A few such immunopathological components of parasitic diseases have been suggested but not as yet established. The nephrotic syndrome associated with *P. malariae* infections is one of these. Here the kidney does not appear to be a primary target of the parasite but the renal injury may rather be secondary to a host antiparasite-antibody response.

In bilharziasis, the finding of marked inflammatory reactions in the lung, of vasculitis in the colonic vessels, and of severe reaction to dead worms in the liver of some immunized animals suggests that further pathological studies are needed to evaluate the role of the immune response of the infected animals in the development of such tissue lesions as, for example, the development of "pipe-stem" cirrhosis.

In order to evaluate the evidence and importance of immunological or hypersensitivity reactions in parasitic diseases, a thorough study of the
immunopathogenetic processes in these diseases will be necessary. The resultant definition of immunopathogenetic events will be important for the understanding both of the events themselves and of the protective immune responses of the host.

5. ANTIGENS OF PARASITES

The complexity of the life cycle of zooparasites is reflected in the complex variety of possible parasite antigens and the equally various mechanisms for their liberation. The antigens of zooparasites are usually classified into two general groups: (a) exogenous, i.e., secretions or excretions liberated by the parasite during growth, development or tissue penetration and (b) endogenous, i.e., "structural" or "somatic" antigens present in the external cuticle and/or internal organs or organelles of the parasite and that may, in some instances, come into contact with the host only upon the death and disintegration of the parasite. The newer physico-chemical techniques for purification and characterization of antigens, which are only beginning to be applied to antigens of zooparasites, may greatly help in unravelling their complexity.

5.1 Liberation during invasion of the host

Antigenic differences occur in the various stages in the life cycles of zooparasites. Some of these differences are qualitative, while others are only in the relative proportion of common antigens. The exposure of a host to antigen may occur almost immediately upon infection, and therefore it is pertinent to consider ways by which parasites invade their host.

Many parasites enter their host passively, being ingested with food or water or being inoculated by the bite of an insect. In helminths, the infective organism can occur in a variety of forms, e.g., as a larva enclosed in an eggshell or in a protective sheath, encysted in the body of an intermediate host, etc. Intestinal protozoa usually enter the digestive tract enclosed in a cyst. The infective organisms within their protective membranes are activated in the digestive tract and escape from the membranes that enclose them. During these hatching, exsheathing or excysting processes, the fluids enclosed within the protective membrane are released into the digestive tract. These include, for example, the metabolic excretory and glandular secretory products that accumulate inside helminth eggs. So far only a few studies have been done to investigate the potential antigenicity of these fluids. It has been reported, however, that at the time of exsheathment of third-stage infective nematode larvae within the digestive tract, the exsheathing fluid liberated contains antigenic material to which the host
responds, and that this may be true of the subsequent moult(s) and exsheaths-
ments that occur as the organism continues its growth and differentiation.

For many parasites, the lumen of the gut serves only as a portal of entry
into the host, and from this area migrations to other parts of the body may
take place. These may be fairly limited, as when a coccidial sporozoite
penetrates into an epithelial cell of the intestine, or a trichostrongylid
nematode larva invades the intestinal wall no deeper than the muscularis.
On the other hand, other parasites may penetrate directly through the gut
wall into the peritoneal cavity, or may enter a small venule or lymphatic
vessel to initiate an extensive migratory cycle that may take them through
various organs, e.g., the liver, lungs, brain. In some instances the migra-
tion is in the form of a circuitous route in which the parasite ultimately
returns to the gut. In this type of migration, which often involves penetra-
tion of connective and other tissue, the parasite may secrete various lytic
enzymes that attack proteins, carbohydrates and lipids. It is possible that
such enzymes may act as antigens. Specific information on this point has
been better established for skin-penetrating parasites, as indicated below.

Parasites may also directly penetrate the intact epidermis, as in the case
of hookworm and schistosome larvae that come in contact with the host
from contaminated soil or water respectively. To accomplish entry into
the host, these organisms possess specialized glands secreting histolytic
substances that facilitate passage through acellular or intercellular areas
of the skin. One of the chief components of these acellular barriers consists
of mucopolysaccharides and glycoproteins. Alterations in these occur
during the penetration of some but not all species of helminths that have
been studied, and appear to be due to the action of the released glandular
secretions. For example, hyaluronidase-like activity has been reported for
the secretions of some helminths (e.g., schistosome cercariae). It should also
be mentioned in this connexion that hyaluronidase as well as proteolytic
enzymes have been found to be liberated by the trophozoites of *E. histoly-
ticus*. A lipase that acts on tripalmitin, a normal skin constituent, has been
demonstrated too in the secretions of the infective schistosome cercariae
and in two species of larval nematodes. The functional significance of these
enzymes with regard to skin penetration has not been clearly established.
Although there is some evidence that lipase activity is inhibited by “immune”
serum, and that a rise in anticollagenase activity occurs in the serum of
schistosome-infected individuals, there is uncertainty as to whether these
activities are truly antibody in nature.

5.2 Relation to parasitic mass, physiology and reproduction

The considerable increase in the amount of parasitic material during
growth, maturation and reproduction, unique to zooparasites, may result
in antigen release of a magnitude and variety far in excess of that seen in
microbial infections. The development of protective immunity, when it occurs, has almost invariably been associated with the presence of complex living, metabolizing organisms; injected or dead organisms or extracts have at least been of lesser value, and frequently have shown no activity. The formation of precipitates at the body openings of helminths incubated in immune serum (Sarle's reaction) has supported the concept that the secretions and excretions liberated by helminths are antigenic and may give rise to protective antibodies. With the development in recent years of in vitro cultivation procedures for some helminths, it has been possible to collect these so-called excretory and secretory products of living worms and to demonstrate some degree of protective immunity by injecting these materials into the host. This has strengthened the view that functional immunity is associated with the products liberated from the viable organism. Among nematodes, for example, the period of moulting may be particularly important in the immune response. Evidence has been presented that during this period there is a release, presumably in the moulting fluid, of antigenic material that is important in the development of functional immunity.

The purification, biological characterization and chemical identification of these antigens is one of the most challenging areas in the field of immunity to zooparasitic diseases.

5.3 Antigenic variation

An important problem associated with parasitic growth and differentiation is that of antigenic variation with change of stage. With successive stages in the life cycles of many parasites, marked differences in metabolism may occur in response to the various types of physiological and biochemical milieu encountered as the parasite invades different cells, tissues, or body fluids, and these differences may be reflected in the production of different types of antigens. In helminths, for example, antigenic differences have been reported between the third- and fourth-stage larvae of Haemonchus contortus, and between the second- and third-stage larvae of Ascaris lumbricoides. Similar differences have been observed for Trichinella spiralis, Schistosoma mansoni and other parasites of man.

A particularly interesting example of another type of antigenic variation is seen in trypanosomiasis.

5.3.1 Trypanosomiasis

In man and lower animals during chronic infections with some species of trypanosomes, a fluctuation of parasitaemia is observed with a periodicity of five to nine days. Corresponding to the parasitaemic fluctuations, there is in the T. brucei subgroup a periodical variation in morphology, short trypanosome types tending to be most common at times between the parasitaemic waves.
In addition to morphological evidence of periodicity, infectivity titration tests of the blood during natural infections in man have revealed a similar periodicity in the infectivity of the blood. Moreover, different parasitaemic waves consist of a series of different and antigenically distinct trypanosome populations, which may be distinguished from one another by agglutination, neutralization and other procedures. The antigenic variants appear to be correlated with the successive parasitaemic waves, each successive population stimulating the production of, and being controlled by, the antibodies produced by the host. Experimentally infected rats often survive the first parasitaemic wave but are overwhelmed by the second, dying with a fulminating parasitaemia. More prolonged infections occur in animals such as rabbits and bovines. In bovines, the sera of infected animals at any given time usually contain agglutinating and neutralizing antibodies to previously — but not subsequently — isolated variants. There is a tendency for variant trypanosome populations to revert to a comparatively constant "parent" antigenic type on cyclical passage through *Glossina*.

Antigenic variation occurs in clones. Twenty or more antigenic variants occurring with a given strain have been recorded. In infections caused by the same strain in different animals there is a tendency for the variants to occur in a probable pattern, certain variants tending to occur early in the course of the infection. Further studies utilizing this observation should allow a clearer definition of these antigenic variations, whose number may be great but not unlimited.

Analysis of the antigenic constitution of trypanosomes indicates the presence of:

(a) antigens released only by disruption of the organisms common to several trypanosome species (and therefore not involved in the determination of antigenic types), and

(b) type-specific antigens released into the ambient fluid (whether by trypanosome breakdown or by normal metabolism is uncertain).

5.4 Shared antigens

Cross reactions between zooparasites of different species, families and even phyla are frequently encountered. Because of these cross reactions, the significance in endemic areas of positive serological reactions to a particular antigen is sometimes difficult to assess.

For example, a cross reaction has been observed between *Schistosoma mansoni* antigens and sera from patients with *Trichinella spiralis* infection; this has been studied by absorption techniques. When the *Trichinella* antiserum is absorbed with *Trichinella* antigen, the homologous antibody is removed but the antibody reaction against schistosome cercarial antigen remains. The converse is also true. Similar cross reactions between these
two antigens using other serological techniques may be avoided by heating
the S. mansoni antigen to 56°C for 15 minutes.

Cross reactions between Salmonella typhi and Trichinella spiralis have
also been reported. Recent studies have shown that the somatic XII
antigen of S. typhi is the antigenic determinant shared with T. spiralis.
Mice immunized with this somatic XII antigen (which has a carbohydrate
specificity of known structure) are protected against challenge with larvae
of T. spiralis.

Other examples of cross reaction include the demonstration of Forssman
antigen in Trichinella spiralis; the observation that infection of animals
with Ascaris lumbricoides produces a marked elevation of heterophile
antibodies reactive with erythrocytes of other species; the stimulation of
high levels of antibodies reactive with human blood group A antigen in
rabbits by infecting them with Ascaris lumbricoides, and of antibodies
against human blood group A and B antigens in pigs infected with Ascaris
suum. Blood group B antigen is found in hydatid cyst fluid. There is,
however, no evidence that high levels of heterophile antibodies or the
possession of antibody against these blood group substances modifies the
course of the parasitic infection.

5.5 « In vitro » cultivation and production of antigens useful for immuniza-
tion

As already mentioned one of the greatest drawbacks, until recent times,
has been the inability to culture parasitic helminths and certain important
protozoa in vitro.

Dictyocaulus viviparus, Trichostrongylus colubriformis, Ascaris and
Strongyloides papillosus can now be cultured in vitro, and have yielded
antigens that were highly effective in protecting guinea-pigs or rabbits
against these infections. In some cases the antigens were relatively stable,
but in others they were quite labile, and a marked reduction in their effec-
tiveness occurred with storage at −20°C. Lyophilization had some deleter-
ious effect on the antigens, but this was not as marked as that from ordinary
storage in the frozen state.

While limited in vitro cultivation of non-human plasmodia has been
achieved by a few workers, no successful technique has been developed
for any of the malarial parasites of man. The need to establish cultures of
erthrocytic and exoerythrocytic forms is urgent. The specificity of
human exoerythrocytic stages for hepatic cells suggests that a prerequisite
for their adequate cultivation may be the establishment of successful
methods of in vitro culture of liver cells.

Cultivation of trypanosomes in vitro may be accomplished in liquid
media, but it appears to permit only the first part of the natural cycle of
development in the vector. The antigens produced by trypanosomes in
culture are not protective on inoculation into animals, but antisera to
cultured trypanosomes are inhibitory to homologous cultural forms.
Metacyclic infective forms have occurred in culture only exceptionally,
though culture forms fed to Glossina may complete their development to
metacyclic forms in the fly. The factors influencing the completion of the
cycle of development are unknown.

6. METHODS

The observations demonstrating the reaction of antibodies with living
zooparasites now allow the application of the full range of immunological
methods to parasitic infections. Although there has been widespread use
of immunodiagnostic techniques because of their practical importance, their
development and effectiveness have been hindered by the fact that little
work has been done to obtain purified antigens.

Most of the antigens employed in immunodiagnosis consist of crude
and undefined preparations (disintegrated whole organisms or extracts).
In recent years, however, more effort has been made to isolate and charac-
terize antigens from a variety of parasites. The isolation of some diagnosti-
cally specific antigens from T. spiralis, A. lumbricoides, E. granulosus
(cyst fluid) and D. immitis has now been reported. As methods for the
isolation of parasitic antigens improve, immunodiagnostic methods should
become increasingly effective tools for the detection of parasitic infections
of man and animals.

6.1 Immunodiagnostic techniques

(a) The complement fixation (CF) test, because of its early introduc-
tion to the serology of bacterial and viral diseases, has been extensively used
in the diagnosis of a number of parasitic infections. While useful results
have been obtained, their interpretation has been difficult because of the
complexity of the crude antigens used and the numerous cross reactions
observed.

In trichinosis, some improvements in sensitivity and specificity have
been reported with antigens obtained from dried T. spiralis larvae by
extraction with ethanol and fractionation under controlled conditions of pH
and ionic strength. An ethanol-soluble (ES) fraction contained glycoprotein
and the ethanol-insoluble (EI) fraction nucleoprotein. Neither contained
detectable lipid. The ES fraction was heat-stable and unaffected by freezing
or lyophilization, whereas the EI fraction lost much of its serological activity
after such treatment. Absorption with ES selectively removed the homo-
logous antibodies, indicating that antibodies against at least two different
antigens were elaborated during the infection. In animal and human
infections with *T. spiralis*, anti-ES antibodies appeared later and disappeared sooner than anti-EI antibodies.

For diagnosis of human filariasis, alcoholic extracts of *D. immitis* have been used and complement-fixing antibodies have been demonstrated in a relatively high percentage (30-50%) of individuals infected with *Wuchereria bancrofti* and *Acanthocheilonema perstans*. Possible reasons for failure to detect all cases by this technique may be the neutralization of circulating antibody by large numbers of microfilariae or failure of sufficient cross reaction to a heterologous species. More recently, promising results have been obtained in the CF test with a purified protein fraction isolated from *D. immitis*, but more extensive evaluation of this antigen is needed.

In trematode infections and particularly bilharziasis (due to *S. mansonii*, *S. haematobium* and *S. japonicum*) the CF test has provided considerable information. This test permits the detection of infection before the worms reach maturity and produce eggs. However, it is not yet possible to differentiate the infections in man produced by these three species. Most cross reactions with unrelated parasites have been prevented by using antigens obtained by delipidation of the parasitic material prior to its extraction. Such treatment of the antigen has also minimized its anticomplementary activity. The CF test in bilharziasis appears to be a valuable aid in the diagnosis of bilharziasis and may prove to be useful in epidemiological surveys and for the assessment of control; further attempts to fractionate and purify the antigenic material and improve the performance of the test are still necessary.

The varying degree of success with which the CF test has been applied to the diagnosis of these and many other parasitic diseases (e.g., amebiasis, toxoplasmosis, cysticercosis, hydatid disease) is presumably due to lack of suitably defined antigens.

*(b) The passive haemagglutination (HA) test* since its introduction has been utilized for the serological diagnosis of a number of zooparasitic infections. In experimental trichinosis this test has detected antibody as early as six days after infection. In human trichinosis, the haemagglutination test appears to be considerably more sensitive than the CF test. The antigen used is generally an acid-soluble protein fraction (Melcher antigen).

In bilharziasis, the reproducibility of passive haemagglutination titres has been poor, and further work to increase the sensitivity of this test will be necessary before it can be used for diagnostic purposes.

In recent years, hydatid cyst fluid antigens have been used in the passive haemagglutination technique for the diagnosis of echinococcosis. In patients suffering from this disease, the HA test with human cystic fluid as antigen was more sensitive and specific than the CF test with the same antigen. Considerable sensitivity of the test based on clinically diagnosed and surgically verified echinococcosis has been claimed. The complexity of
the antigen preparations used still represents a considerable problem. It is of interest that cyst fluid of the related cestode, _Taenia hydatigena_, appears to have a cross-reacting antigen in the HA test for hydatid disease.

The HA test is also used with some success for the diagnosis of amoebiasis. The antigen used has been a water extract of the disintegrated organisms collected from a culture of _E. histolytica_ in Phillips' medium with _Trypanosoma cruzi_ as the associate. It has been reported that sera from 93% of individuals with symptoms of amoebiasis and passing _E. histolytica_ cysts give positive haemagglutination tests.

In toxoplasmosis, reproducible results have been obtained using as antigen a water extract of washed _T. gondii_ obtained from peritoneal fluids of infected mice. This test appears promising, but further evaluation is needed.

(c) The precipitin test (double diffusion in agar gel) has been widely used for the detection of precipitins in most infectious diseases, as well as zoonotic parasitic infections. In experimental infections in animals, precipitins were detected as early as six days after infection in trichinosis and five days after infection in ascariasis. Experiments using purified antigens from _Trichinella spiralis_, _Ascaris lumbricoides_ and _S. mansoni_ have given results in the past few years indicating that the agar diffusion test is a potentially useful immunodiagnostic tool.

(d) The semi-quantitative or quantitative determination of immunoglobulin levels by agar gel diffusion methods may give useful information. In Senegal, it has been shown that patients with trypanosomiasis have significantly higher levels of γM globulins in their sera than normal subjects and patients with most other diseases. The sera of some individuals contain more than ten times the normal level of γM globulin. This test is the subject of intense investigation because of its potentially great usefulness in the epidemiology of trypanosomiasis. It also seems to serve as an index of therapeutic effect. A patient who, after therapy, still has a high level of serum γM globulin will probably relapse. The demonstration of γM globulin in the cerebrospinal fluid of patients in Senegal is accepted as specific evidence of central nervous system involvement in trypanosomiasis. Tests have recently been developed for the field determination of γM globulin in the cerebrospinal fluid. These studies are of great interest because of the need for a method of diagnosis of trypanosomiasis in the numerous patients in whom it is impossible to demonstrate the parasite.

(e) The immediate type of hypersensitivity is one of the features of most zoonotic parasitic infections. The observation of this manifestation of parasitism led the way to the application of intradermal tests for diagnosis of a number of infections. Because of non-specific reactions due to the variety of crude and undefined antigens that have been used for many years, the results obtained have had only relative diagnostic value. However, these
tests have been used widely in the epidemiological assessment of infections such as bilharziasis, hydatid disease and trichinosis. In 1960, WHO began a comprehensive programme for the comparative evaluation of existing schistosome skin tests. Nevertheless, the degree of reactivity and its relationship to active, acute, chronic or cured infection are not known and require further investigation.

(f) The fluorescent antibody test, particularly the indirect method, appears to be one of the most promising serological techniques used in diagnosis of zooparasitic infection. This test has been used for diagnostic purposes in malaria, toxoplasmosis, schistosomiasis, trichinosis and more recently in trypanosomiasis and filariasis. The indirect technique as applied to bilharziasis has been improved and non-specific reactivity of the cercariae diminished by the use of rhodamine bovine albumin (RBA) as a counterstain. This method obviates the need for maintaining a fresh supply of cercariae, and permits satisfactory results even with cercariae which have been frozen, lyophilized or preserved in formalin. The application of this test to epidemiological studies became more practical after the discovery that drops of blood obtained by finger prick, dried on filter-paper, and mailed to a central laboratory could be extracted and used successfully in the fluorescent antibody test. These two improvements make the fluorescent antibody test a simple and practical one for the field diagnosis of bilharziasis, and bridge the usually conspicuous gap between field diagnosis in endemic areas and the availability of central diagnostic laboratories.

6.2 "In vitro" methods for the detection of hypersensitivity

6.2.1 In vitro methods for measurement of delayed hypersensitivity

The addition of antigen causes a decrease in migration of macrophages from lymph nodes, spleen and lung fragments in tissue culture, or of macrophages of human buffy coat cells packed into capillary pipettes when the tissues or macrophages are obtained from animals exhibiting delayed hypersensitivity reactions to the antigen. This method should be applied to the study of delayed hypersensitivity to antigens of parasites in the parasitic diseases.

6.2.2 The application of in vitro lymphocyte transformation

It is known that many macromolecular substances added to in vitro cultures of peripheral blood lymphocytes can cause some of these cells to divide and differentiate. It has been claimed that such cells may synthesize immunoglobulins.

The response to certain agents (e.g., phytohaemagglutinin and streptolysin S) is shown by the majority of lymphocytes of normal individuals,
and appears to be immunologically non-specific. The possible significance of other exogenous or endogenous agents should be investigated, in relation to the development of massive immunoglobulin production in certain diseases, notably visceral leishmaniasis and trypanosomiasis.

In the case of most other substances found to be active, the response is shown by lymphocytes of some individuals but not of others, and the proportion of lymphocytes involved may be small. The cell donor usually shows evidence of a pre-existing immunological reactivity to the agent involved. In such instances the in vitro transformation reaction has been taken as indicating that the active agent is an antigen and that the cells are specifically reactive to it. Although the precise nature of such reactivity is not yet known, in vitro lymphocyte transformation is of great interest, and study of this kind of response using parasite antigens should be encouraged.

6.3 Standardization of inoculum and challenge dose

The reproducibility and comparability of experimental results necessarily depend on accurate control of the infective inocula used, on quantity and quality, and on route of administration. There are several ways in which parasite materials may be kept for experimental use over long periods of time: by serial passage in laboratory animals, by serial passage in cultures, and by preservation in viable form. In the two first methods, reproduction of the parasite introduces the danger of mutation which may alter the parasites. The term "stabilate" has been introduced for parasites preserved by low-temperature preservation methods; it is likely that these methods can be adapted to all protozoan parasites.

6.3.1 Trypanosomes

Satisfactory methods for low-temperature preservation of stabilate trypanosome material have been developed. Capillary tubes containing trypanosome suspensions in 7.5% glycerol are cooled to −60°C during a period of no less than 8 minutes, and stored at −80°C. Blood, culture and metacyclic forms have been preserved in this way and stability of such material over 900 days has been established.

The relationship between trypanosome numbers and infectivity is not constant. In most experimental parasitological contexts, the latter will be the more significant parameter. After the infectivity of a stabilate has been established by titration, it may be used repeatedly to provide the desired challenge. By the substitution of individual cultures for animals as recipients of the test doses, the method may equally be used to measure the infectivity of suspension to cultures.
6.3.2 *Helminths*

The antibody response to graded doses of *S. mansoni* cercariae has been determined in different species of animals. Antibodies were detected (fluorescent antibody technique) in Rhesus monkeys as early as 3-4 weeks after infection. The time interval between exposure and the initial appearance of antibodies was inversely related to the number of cercariae to which the monkeys were exposed. Similar results were obtained in albino mice; antibodies were detected as early as five weeks and appeared to be related to the number of cercariae in the inoculum.

In helminth infections, the number of larvae in the inoculum producing immunity is often of importance. Similarly, the number of larvae in the challenge dose frequently determines whether or not protective immunity is adequate. This relationship in bilharziasis has been well worked out for serological responses and for protection to challenge.

In *Dictyocaulus* infections, 25% of the inoculum can regularly be recovered. Once the inoculum goes above 5000 larvae, however, the percentage recovered is greatly reduced; when more than 12,000 larvae are given, most calves die before adult worms develop. The number of larvae in the inoculum is also important in studies of immunity. One hundred larvae produce no significant protection, 1000 substantial protection, and 4000 complete protection against infection in the 75% which survive the lung lesions developing in association with the immune response (see section 3.2.6).

Similar results have been demonstrated in other helminth infections such as by *Haemonchus, Ostertagia* and *Ancylostoma*.

6.4 Action of antibody on parasites “in vitro”

*In vitro* tests present an opportunity for the study of the mechanisms of action of antibodies upon parasites. When a living parasite is treated with antibody, observations can be made (microscopically or macroscopically) of agglutination, immobilization and lysis. In some cases a precipitation reaction may be observed around the parasite (e.g., *Trichinella spiralis* larvae). In addition, modifications in metabolism of parasites may be detected and measured by the aid of biochemical techniques (e.g., Warburg manometry).

Some of these methods have been used as diagnostic tools. But these techniques have a number of practical disadvantages which limit their application. Living parasites are essential and are usually obtained by *in vitro* cultivation or passage in laboratory animals. In both cases there is a serious risk that the parasites may have characteristics quite different from those in the normal host. Moreover, the agglutination, immobilization and lysis tests often show a strain specificity, and therefore several strains must
be tested before concluding that a test serum contains no antibodies against the parasite in question.

6.5 The use of germ-free animals

The value of the germ-free animal in immunological research derives from the fact that it contains no living microbial flora or fauna to serve as antigenic stimuli. The only known antigens to which these animals are exposed are the components of the diet and perhaps viruses transmitted in utero, although they have yet to be demonstrated. Therefore their antigenic experience is minimal as compared with conventional animals. This is reflected by their relatively low number of immunologically competent cells, and their low level of gamma globulin.

The few parasitological studies performed to date have not been immunological, but rather have dealt with tissue invasion and pathogenicity. Early studies with Entamoeba histolytica injected along with Trypanosoma cruzi into the caecum of the germ-free guinea-pig demonstrated that the amoebae, in the absence of an associated bacterial flora, rapidly disappeared from the lumen, and that only a few organisms maintained themselves in the traumatized tissue at the site of inoculation. No evidence of intestinal lesions or ulcerations occurred. In more recent studies, in which the inocula were processed to exclude the deleterious effects of oxygen, higher numbers of amoebae were found in the caecal tissue, but, again, no lesions typical of amoebiasis in the conventional guinea-pig occurred. However, when amoebic inocula were introduced into guinea-pigs that had been monocontaminated with bacteria e.g., Escherichia coli or Aerobacter aerogenes, acute ulcerative amoebiasis occurred, and was nearly comparable to that seen in conventional control animals. On the other hand, the development of T. cruzi in the germ-free guinea-pig appeared to be more extensive than in its conventional counterpart.

In studies with helminths that would be considered abnormal parasites for the guinea-pig, it was found that in the germ-free animal, the rat nematode Nippostrongylus brasiliensis (= muris), the mouse nematode Nematospiroides dubius and the mouse tapeworm Hymenolepis nana all developed to normal fertile adults. While worm yields in the case of N. brasiliensis were very low, in the case of N. dubius they were as high as 23% of the larvae inoculated. Development of these helminths in conventional guinea-pigs was either extremely poor or failed to occur. Of interest was the fact that larvae of N. dubius that penetrated the intestinal wall were rapidly surrounded by an inflammatory response in the germ-free guinea-pig. This observation contradicts the suggestion that similar inflammatory reactions seen in conventional animals might be due to accompanying bacterial invasion, and not to the nematode.
N. dubius has also been established under germ-free conditions in its normal host, the mouse, and has been successfully carried through several germ-free passages. It thus appears that germ-free animal-parasite systems are feasible tools and may be profitably utilized in the future to study both parasite metabolism and immunological phenomena.

7. RECOMMENDATIONS

A. Immunopathology

In view of the probable importance of the immunopathology of the parasitic diseases, increased emphasis on research in this area is needed. In order to serve as a significant stimulus to this research, it is therefore recommended that a meeting on the immunopathological and pathogenetic aspects of parasitic diseases be planned by the WHO.

B. Antigens

The logical and probably necessary first step toward achieving immunological measures of host resistance and immunological means of inducing or increasing that resistance is the definition of antigens related to critical stages of parasite development. Field testing with crude preparations of multiple antigens is not likely to add significantly to our knowledge of the pathogenesis of parasitic diseases. If essential components of the parasite, preferably those in contact with the host environment, can be isolated and found antigenic, they would serve as the best possible test and immunizing agents. The urgent necessity for such studies has emerged from this discussion in many places in the report and especially in the following areas:

1. the differentiation in various parasitic diseases between
   (a) non-protective antigens (which may be useful in diagnosis), and
   (b) protective antigens (necessary in immunoprophylaxis and possibly also in diagnosis);

2. the search for antigens common to parasites and the tissues of their particular host species;

3. the chemical nature of the antigens secreted at natural orifices in helminths, as these may possibly be involved in protective immunity;

4. antigenic analysis of moulting fluids of helminths;

5. morphological and physiological studies in helminths that would lead to identification of antigenic structures, e.g., in the cuticle;

6. the characterization of anti-enzymes, inasmuch as parasite enzymes are important in penetration and migration.
C. Antibodies

In view of new knowledge of heterogeneity among antibodies and the different biological effects (skin-sensitizing, anaphylactic, complement-fixing, etc.) of various types of antibodies, it is increasingly important to determine which of the antibodies detected in current tests for diagnosis of parasitic diseases are effective in immunity against parasites. For example, there is urgent need for further evaluation of the suggestion that skin-sensitizing antibodies may prevent the penetration of schistosome cercariae.

In persons with parasitic infections that cause a marked increase in immunoglobulin production, it appears that the immunoglobulin increase is only partly explicable by the synthesis of specific antibodies against antigens of the parasite, and the nature of the remainder is unknown. It is important to investigate the immunological responsiveness of such persons to antigens unrelated to those of the parasitic agent. The extent and time-course of the antibody response to both polysaccharide and protein antigens should be studied.

D. Malaria

The Committee notes that despite immediate, formidable obstacles, the prospects for immunization against malaria are promising and recommends:

1. renewed activity in the fields of in vitro cultivation of all phases of the parasite;

2. study of the general antigenic composition of plasmodia and identification of the antigen(s) reacting with fluorescent antibody;

3. investigation of the effect of pregnancy upon the maintenance of acquired immunity;

4. carefully controlled experimentation in human volunteers and in monkeys in non-malarious environments

(a) to ascertain the minimum infective doses of sporozoites and/or asexual erythrocytic forms in primary infections and in subsequent challenges with parasites derived from the same source, and

(b) to determine the possible importance of antigenic difference between species and between strains within a given species (This may be done in the following way: individuals who have recovered from a single infection induced by a standardized inoculum of parasites derived from a given source should be challenged with inocula of the same magnitude derived from the same or from several different sources. The observation of significant differences both in the course of clinical illness and in the duration and density of asexual parasitaemia would provide presumptive evidence of antigenic dissimilarity. In the human volunteers
the existence of genetic peculiarities of the erythrocytes, e.g., haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency, should be sought routinely);

(5) investigation of factors that may influence the infectivity of gametocytes in mosquitoes;

(6) determination of the role and importance of immune mechanisms in the production of malarial anaemia.

In view of the relative unavailability of human volunteers for studies with *P. falciparum* and because of the fact that any individuals must, for ethical reasons, be treated before they develop high parasitaemias, it is recommended that studies be encouraged to determine whether splenectomized chimpanzees can be used successfully for immunological studies of human malaria.

E. *Tripanosomiasis*

In view of the vital need to secure control of trypanosomiasis in man and in animals in Africa, and of the difficulties in the way of vector eradication and chemotherapeutic approaches, intensive investigation should be made of the possibility of developing immunization procedures for this purpose.

1. A further study of antigenic variation in trypanosomes is required:
   (a) to establish the number of antigenic variants which may occur within the same material, both in the vertebrate host and in the insect vector;
   (b) to find out the nature of the specific antigenic determinants involved in these transformations and evaluate their significance (it should be noted that current concepts of antigenic variation in trypanosomes are derived practically exclusively from studies on *T. brucei* subgroup organisms; special attention should be devoted to immunological studies related to *T. vivax* and *T. congolense*, probably the most important trypanosomes affecting cattle in Africa, to discover if they behave similarly).

   Although evidence has been produced to indicate that the antigenic variant types of trypanosome, arising in succession in chronic infections in the mammal, revert to "parent" basic types on cyclical transmission through *Glossina*, it is not known how many basic types exist in any given area in Africa. Special studies should be made to determine this information for at least two areas in both East and West Africa.

2. Certain African breeds of cattle, notably the N'Dama, exhibit a capability to adjust to trypanosome infection without clinical signs that is
close to that attained by most wild African ungulates and conspicuously different from the susceptibility of other breeds, particularly those most to be desired economically. These cattle breeds, therefore, offer very favourable subjects for the study of the immune mechanisms determining this capability. The Committee recommends, therefore, comparative study of the immune responses of these breeds with those of more susceptible breeds.

3. Because of the virtual prohibition usually imposed by quarantine regulations on experimental study of African pathogenic trypanosome infections in large animals outside Africa, there is urgent need of an approximately similar model for experimental, immunological and epidemiological study in temperate climates. Such a model would not only be of greatest use as a research tool but would offer problems suitable for the introduction of young research workers to the subject. There is evidence that Trypanosoma theileri (Stercoraria) exists as a frequent infection of cattle in temperate Europe and elsewhere, usually escaping notice, however, because of its occurrence at extremely low, almost invariably non-pathogenic, levels of parasitaemia. Trypanosoma theileri, therefore, seems likely to offer a particular favourable model for epidemiological and immunological study, and the Committee recommends that it should be intensively studied to determine its suitability for the purposes outlined.

4. There is a great need for a convenient method of distinguishing the morphologically identical species, T. rhodesiense (man-infecting) and T. brucei (non-man-infecting). Immunological studies may provide such a method; to facilitate such studies a specific effort should be made to establish a series of T. brucei subgroup materials as non-man-infecting and to make these materials available as stabilates to research workers.

5. In order to improve the reproducibility and comparability of results, the Committee recommended that whenever possible experimentation should be made with e.g., trypanosomes stored in the frozen state for which comprehensive documentation is available, and many other "microbial" parasites. Banks of such materials should be established. The materials made available should include:

(a) clones, set up from single blood forms, and

(b) metacyclic trypanosomes from Glossina infected from clones.

Similar methods of storage should be applicable to other unicellular parasites and the possibilities for storing metazoan parasites in the frozen state should also be explored.

6. Tissue cultures should be tested for their ability to support the growth of protozoan parasites. In the case of trypanosomes, tissue cultures from arthropods might be tried.
7. The special tendency of trypanosome antigens to be associated with extremely elevated γM serum antibody levels should be investigated.

F. Unclassified

1. Infectivity titration methods have shown that the infectivity of a suspension of parasitic organisms, such as trypanosomes, is not necessarily constantly related to the concentration of organisms contained in it. From the recognition of the independence of these two parameters it follows that, wherever possible, both should be estimated; in many contexts the parameter of infectivity will be the more significant.

2. In view of the fact that in many parasitic infections it is difficult to establish a diagnosis either by clinical methods or by detection of parasites, it is recommended that:

   (a) field investigations be conducted in different endemic areas to evaluate newly developed serological techniques requiring minute amounts of blood for use in field operations (examples of these techniques are the plasma card test and the fluorescent antibody test, using blood dried on filter paper); and

   (b) that these methods be applied in comparing the sensitivity and specificity of the purified preparations of antigens as they become available, and at present of the recently recognized exo-antigens with existing somatic antigens.

3. Progress should be hastened towards evolving irradiated vaccines usable in man, particularly against hookworm and other important intestinal parasites. Further attenuation and irradiation studies should be made on the Formosan strain of schistosome and other strains of minimal pathogenicity, towards the same end.

4. Germ-free techniques should be exploited to study antibody and cellular response in animals infected with a single parasite species.

5. Investigation should be undertaken of the possible importance of specific immunological unresponsiveness (paralysis) in those diseases in which very large numbers of parasites are present in the tissues over prolonged periods of time but no immunity is developed (e.g., infections with *Leishmania donovani* or *Wuchereria bancrofti*), or in which effective immunity develops in response to immunizing agents under some conditions but not under others (e.g., in partial immunity to *Haemonchus contortus* in lambs).

6. There is urgent need for methods to be devised for the *in vitro* cultivation of parasites through all the stages in their life cycles in defined media. Under such conditions, antigens occurring at different points in
development could be studied critically for possible variation quantitatively and qualitatively. The possibility would also be present of collecting antigen in large quantities for chemical fractionation and immunological characterization. Such studies may make possible the identification and separation of protective antigens from antigenic extracts that also stimulate the production of antibody not concerned with protective immunity. The use of such materials as vaccines should be explored in detail.

7. It is recommended that parasitologists should be encouraged, with WHO support, to work in immunological laboratories and immunologists in parasitological laboratories in endemic areas, to ensure that both sides become fully acquainted with one another’s methods.

8. To permit investigations into immunity against parasites by individuals who have limited access to parasite materials, it is recommended that arrangements be made for the collection, storage and distribution of parasite materials and antisera, in freeze-dried or other stable form, for biochemical and immunological analysis outside the endemic areas.

9. The Committee gave high priority to the programme for WHO Immunology Training and Research Units in developing countries, one example being the newly organized prototype unit at the University of Ibadan, Nigeria. The programme is based on providing training in research by bringing scientists to the developing countries, in order to avoid the ill-effects due to geographical dislocation of the scientist-in-training, which too often include failure to return to his native country.

The programme of training is designed to give broad modern theoretical and experimental knowledge of research trends and techniques, with a view to stimulating creativity and a level of technical skill suitable for the most advanced attack on fundamental and practical problems of medicine and allied sciences.

In addition, the eminent scientists who will undertake the training and research will be brought at the same time into direct contact with the pressing health problems of the developing countries, inter alia the parasitic diseases. This can be expected to increase the number of immunologists who will take up problems directly or indirectly related to the parasitic diseases.

ACKNOWLEDGEMENT

The Committee acknowledges the special contributions made during its discussions by the following members of the WHO Secretariat: Dr N. H. Kent, Parasitic Diseases; Dr Z. Trnka, Immunology.