THYMUS DEPENDENCY OF THE IMMUNE RESPONSE IN MURINE MALARIA

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Anil N. Jayawardena

London School of Hygiene
and Tropical Medicine

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ABSTRACT

Studies were carried out to elucidate the role of the thymus and thymus derived lymphocyte population in the immune response of mice to malaria.

Infection of normal CBA mice with the malaria parasite P. b. yoelii resulted in a mild, non fatal infection which lasted 15 to 16 days. Mice which recovered from a primary infection were immune to reinfection. Parasites could be demonstrated in the kidneys of such mice 3 to 4 weeks after recovery from infection, but were not detected in the peripheral blood and other tissues examined.

In T cell deprived CBA mice P. b. yoelii infections followed a fulminant course and proved fatal within 30 to 35 days, thus demonstrating the absolute thymus dependency of these infections. In ATS treated mice P. b. yoelii infections followed an essentially similar pattern, but 30% of the treated mice recovered. Studies of fluorescent antibody levels and the histopathological changes in the spleens of normal and T cell deprived mice during infection showed that while normal CBA mice made high levels of IgG_1, IgG_2 and IgM antibodies and showed a strong and sustained germinal centre response, in T cell deprived mice the production of IgG_1 antibodies was almost completely abolished and the germinal centre response was severely impaired. Reconstitution of T cell deprived mice with syngeneic thymus grafts resulted in restoration of immune responsiveness; the level of protective immunity and the germinal

centre response correlated well with the degree of reconstitution achieved.

The response of the CBA mouse to P. b. berghei, a progressive infection which proved fatal in 20 to 25 days, was also examined. These infections were found to provoke antibody and germinal centre responses intermediate between those elicited by P. b. yoelii infections in normal and T cell deprived mice.

The T cell mitotic response to P. b. yoelii and P. b. berghei was defined in CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras carrying a chromosomally distinguishable population of T cells. While P. b. yoelii provoked a rapid and sustained burst of T cell mitosis, the T cell response evoked by P. b. berghei was feeble and failed early in the infection despite the progressive parasitaemia. The functional state of the T cell pool was assayed by examining the responses of infected CBA mice to Oxazolone and of spleen cells to PHA (Phytohaemagglutinin). P. b. yoelii infected mice responded normally to oxazolone and despite a decrease in the proportion of PHA responsive cells there was a net increase in the absolute number of such cells in the spleen. In contrast, P. b. berghei infections resulted in marked depression of the response to oxazolone and of the absolute number of PHA responsive cells in the spleen. Both infections caused a decrease in the proportion of LPS responsive (B) cells, but when the response was computed on a per spleen basis there was no significant impairment of the response.

Mechanisms of resistance in P. b. yoelii infections were

further analysed by carrying out a series of cell and serum transfers from immune to non immune mice. While hyperimmune serum was protective against low parasite innocula, immune serum had no protective effect. Doses of hyperimmune serum which protected intact recipients completely, caused orly transient inhibition of infection in T cell deprived recipients. Immune spleen cells were the most effective in transferring immunity to normal and T cell deprived recipients. Treatment of these cells with anti theta antiserum did not remove their protective effect, suggesting that the effector cells in the immune spleen cell population were non T cells.

These results are discussed in relation to their significance in effector and suppressor responses elicited during malarial infections.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The last 15 years have seen a revolution in Immunology; a revolution initiated by the demonstration of the role of the thymus (Miller, 1961, 1962; Good et al., 1962), and which gained momentum with the delineation of the two lymphocyte concept of immunity. The relevance of the fine study of the immune response to the problems of tissue transplantation, malignant and infectious disease, has provided the impetus to the revolution. There has certainly been a tremendous surge of activity in the fields of transplantation and tumour immunology, but the "Golden Age of Immunology" has made only a limited impact on the study of infectious disease, particularly the "classical" parasitic diseases. This investigation was initiated in an attempt to remedy the latter situation; it focusses attention on probably the most important of these diseases - malaria.

The interaction between a developing parasite such as a malaria parasite and the immune system must necessarily be complex, and although in recent years there have been significant contributions made towards an understanding of this interaction, the fundamental cellular events involved in the host response are still poorly defined. Malarial infections are also known to depress host reactivity to certain other antigens, and it has been suggested that a causal relationship exists between malaria and Burkitt's lymphoma. This study was aimed primarily at obtaining a greater understanding

of these aspects of the host parasite interaction, in particular the role of the thymus and thymus dependent immune mechanisms; mechanisms, which Burnet (1967, 1970) suggested were evolved primarily as a surveillance system against neoplasia, but which are probably equally important in defence against infectious agents.

The approach adopted in studying the interaction between host and parasite was to "dissect out" the response of the host (the CBA mouse) to the parasite. As the immunology of the mouse is far in advance of any other species, and as the responses of the inbred CBA mouse in particular have been investigated extensively (Davies, 1969), it provides a very valuable system for analysing the immune response to an infectious agent. Use was made particularly, of methods of lymphocyte depletion, reconstitution, and separation to study the actions and interactions of thymus dependent and independent cell populations in these infections.

That the approach used was successful, and that the model holds tremendous potential are borne out by the results presented in this thesis.

CHAPTER 1

THE THYMUS AND THE IMMUNE RESPONSE TO MALARIA PARASITES: A REVIEW.

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SCOPE OF REVIEW:

This review will be concerned essentially with our present understanding of the nature and mode of action of the thymus and thymus dependent lymphocyte population, and its role in mechanisms of resistance to infectious agents in general, and malaria parasites in particular. It is not intended to be a comprehensive review of the subject, but rather an attempt to place this study in perspective, and to discuss those aspects of the literature necessary for an appreciation of the investigative procedures adopted.

SECTION I. THYMUS DEPENDENT AND INDEPENDENT LYMPHOCYTES AND IMMUNE RESPONSES

Two systems of immunity protect the body from the hazards of infection and cancer. The first is the cell mediated response which initiates the rejection of foreign tissues and tumours and is important in defence against viral infection, the other is the humoral response which is effective in bacterial infection and viral re-infections. Although these two mechanisms are not entirely independent, there being considerable interaction between them, they are distinct.

1. THE TWO LYMPHOCYTE CONCEPT OF IMMUNITY

The first evidence for such a functional duality in the immune system, came from the observations of Chase and Landsteiner in the early 1900's. They showed that while some kinds of immune reactions

could be transferred only by cells (lymphocytes), others could be transferred only by serum antibodies. Proof that this functional dichotomy in the immune system did in fact have a developmental basis, came from a series of classic experiments carried out in the early 1960's - the most important of which was the demonstration that removal of the thymus from a newborn mouse consistently impaired homograft rejection and had a variable effect on antibody production (Miller, 1961, 1962; Good et al., 1962; Martinez et al., 1962). Similar effects were demonstrated in rats by Jankovic et al. (1962).

A series of experiments carried out on chickens at more or less the same time, led to a more precise definition of the role of the thymus. As early as 1956, Glick had demonstrated that removal of the bursa of Fabricius (a cloacal lymphoid organ unique to birds) impaired the capacity of chickens to produce serum antibodies, while Warner (Warner et al., 1962; Warner and Szenberg, 1964) found that although surgical thymectomy impaired homograft rejection, inhibition of bursal development had no such effect. He concluded that a functional dissociation existed in the chicken lymphoid system - a concept extended by Cooper (Cooper et al., 1966) who in a series of elegant experiments obtained convincing evidence for a dichotomy in the immune system; the bursa and bursa derived cells being responsible for antibody production, the thymus being concerned with cell mediated responses and having a variable influence on antibody production depending on the antigen used.

The results of these extirpation experiments in birds and rodents were complemented by important clinical observations made by Good and his colleagues (reviewed by Good et al., 1971). They defined a complete spectrum of immunological deficiency diseases - 'the experiments of nature' in which either cell mediated immunity or humoral immunity, or both, were absent.

The two component concept of immunity is now clearly established; the "central" lymphoid organs, the thymus and bursa, producing lymphocytes which seed the "peripheral" lymphoid organs, spleen, lymph nodes and gut associated lymphoid tissue. In mammals no equivalent of the avian bursa has been identified, and it now seems likely that the source of the haematopoietic stem cells, the foetal liver in the embryo and the bone marrow in adults, is also the site of differentiation of bursa equivalent cells. In the peripheral lymphoid tissues, cells derived from the thymus have been termed T cells and those from the bursa or its mammalian equivalent B cells (Roitt et al., 1969).

2. DIFFERENTIATION AND DISTRIBUTION OF T AND B CELLS

2.1 Differentiation

Both T and B cells arise from common ancestral haematopoietic stem cells. These stem cells are found in the yolk sac and foetal liver in embryonic life from where they migrate to, and settle permanently in the bone marrow. The pattern of development which these cells assume is dependent on influences exerted within the microenvironment into which they migrate.

Stem cells which migrate into the thymus differentiate into thymocytes. They then undergo further differentiation into mature thymus lymphocytes after which they move out to the peripheral lymphoid tissues where they assume the characteristics of peripheral T cells. This migration to the peripheral tissues begins in the first week of life (Owen, 1972) hence neonatally thymectomized mice grow up with a marked deficiency of T cells. Thymectomy by itself, later in life, has much less effect. Most thymus lymphocytes are immunologically incompetent and only a small proportion of medullary cells (2-5%) are immunocompetent and possess most of the properties of peripheral T cells.

There is now considerable evidence for heterogeneity within the peripheral T cell population as well. Evidence for functionally distinct sub populations came from the work of Cantor and Asofsky (1972) who showed that two types of T cells acted synergistically in graft versus host (GVH) reactions in mice; the two cells differed in distribution, recirculatory characteristics and thymus dependence. One cell (T_1) was present mainly in the spleen, did not recirculate and was markedly depleted two to six weeks after adult thymectomy. The other (T_2) showed the distribution and recirculatory properties of typical T cells, was not depleted soon after adult thymectomy, but was very sensitive to the effects of ALS in vivo.

On the basis of these observations, they proposed a model (Cantor, 1972; Raff and Cantor, 1971) in which T_1 and T_2 cells

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belonged to the same cell line, the T_1 cell being an earlier stage of differentiation than the T_2 cell. They suggested that the differentiation of T_1 to T_2 was driven by specific antigen and usually took place in the spleen, although T_1 cells if they encountered antigen in the thymus, might differentiate there. On differentiation T_2 cells entered the recirculatory pool.

The T cell component of an immune response after primary or secondary immunisation was thought to depend on the more mature T_2 cell, the response of the T_1 cell being limited to differentiation into a T_2 cell. Enhanced secondary responses were interpreted as being essentially a consequence of large numbers of T_1 cells being driven to the T_2 stage of differentiation in the primary response. Studies on the effect of adult thymectomy and ALS treatment on primary and secondary humoral responses, have largely confirmed the validity of this model (reviewed by Cantor, 1972).

The pattern of B cell development has been shown to be essentially similar, in that stem cells migrate to the bursa where they differentiate into lymphocytes in one to two days. Initially, these cells express IgM on their surface and later IgG. The bursal lymphocytes then migrate to the peripheral lymphoid tissues. Embryonic bursectomy thus results in a marked depletion of these peripheralised B cells and impairment of antibody production.

Until recently it was assumed that B lymphocytes formed a homogenous class of immunocompetent cells, but Playfair and Purves (1971) have presented evidence favouring the existence of

more than one type of B cell (B_1 and B_2). B_1 cells are thought to occur preferentially in the bone marrow and can respond directly to some determinants on antigens such as SRBC. B_2 cells are thought to be located mainly in the spleen and can be triggered to synthesize antibodies in the presence of helper T cells. Whether B_1 and B_2 cells are distinct classes of lymphocytes, B_1 synthesizing IgM and switching to IgG production has not yet been determined. Support for the concept of sub populations of B cells has also come from the work of Weigle et al. (1972), who have shown that spleen and marrow B cells show marked differences in sensitivity to corticosteroids, and in the time required for tolerance induction.

2.2 Distribution

Once T and B cells move out into the peripheral lymphoid organs they localise within distinct areas of the lymphoid tissue. These T and B cell compartments were first recognised by Waksman et al. (1962) in rats, and Parrott et al. (1966) in mice. They found that thymectomized animals had selective areas of depletion in the periarteriolar regions of the splenic white pulp, the mid and deep cortex of lymph nodes and interfollicular areas of gastro-intestinal tissue. These areas were termed 'thymus dependent' areas by Parrott et al. (1966) while the regions unaffected by thymectomy - follicles and peripheral regions of the splenic white pulp, lymph follicles and medulla in lymph nodes, and the follicles in gastro-intestinal tissue - were termed 'thymus independent'.

The existence of these areas, and the ability of T and B

cells to recognise them has been confirmed by cell traffic studies in which radio-labelled cells injected into mice and rats have been shown to migrate preferentially to T and B dependent areas of peripheral lymphoid tissues (Parrott and de Sousa, 1971; Howard et al., 1972; Mitchell, 1972).

The concept of functionally distinct T and B areas was further strengthened by the observations of Davies et al. (1969a, 1969b, 1970). They found that while antigens which evoked cell mediated responses such as Oxazolone triggered off proliferative responses mainly in T areas those antigens which evoked humoral responses caused changes in T independent areas.

3. PROPERTIES OF T AND B LYMPHOCYTES

3.1 Recirculation and life span

Gowans (1959) demonstrated that a considerable proportion of the lymphocyte pool recirculated continuously between blood and lymph. The functional significance of this massive recirculation is still incompletely understood, but recirculation would seem to be an efficient mechanism for ensuring that specific lymphocytes came in contact with antigen and could also serve to disseminate primed lymphocytes throughout the body.

Much evidence has accumulated suggesting that most recirculating lymphocytes are T cells. Experiments with rodents have established that procedures which deplete T cells - neonatal thymectomy, adult thymectomy irradiation and reconstitution with

bone marrow, or ALS treatment, cause a pronounced reduction in the number of recirculating cells recoverable from the thoracic duct (Miller and Mitchell, 1969; Tyler et al., 1968). Further, 80 to 90% of these cells have been found to carry the theta antigen (a marker for T cells) (Raff, 1971), and have also been shown to home preferentially to the 'thymus dependent' regions of peripheral lymphoid tissue (Gowans and Knight, 1964). However, the observations of McGregor and Gowans (1963) that prolonged thoracic duct drainage has no effect on secondary T dependent humoral responses implies that some T cells must lie outside the recirculatory pool.

The nature and extent of B lymphocyte recirculation is controversial. While Sprent and Miller (1972) have shown that T cell deprived mice have relatively few recirculating cells, Howard (1972) has demonstrated that, in rats, B cells recirculate on a comparatively large scale. It seems likely that, although B cells do recirculate, the proportion of such cells is much less than of T cells. Further, they appear to recirculate at a much slower tempo than T cells (Howard, 1972; Sprent, 1973, 1974).

The pattern of lymphocyte recirculation appears to be influenced by various factors. Immunisation for instance, has been shown to cause a non-specific trapping of lymphocytes (Zatz and Lance, 1971); also, soon after immunisation, transient antigen specific unresponsiveness due to selective removal of certain lymphocytes has been observed (Sprent et al., 1971), while McGregor et al. (1971)

and Koster et al. (1971) have shown that, if activated T cells from Listeria monocytogenes infected rats are injected into syngeneic recipients, the cells do not return to the thoracic duct, but migrate into inflammatory exudates. A greater understanding of mechanisms of this sort might prove to be important in explaining the altered patterns of lymphocyte movement found in animals responding to infectious agents.

Lymphocytes are also heterogenous in respect of life span and consist of short and long lived populations. Although T cells have often been equated with long lived cells and B cells with short lived cells, studies on rodents suggest that both T and B cell pools contain similar proportions of long and short lived cells (Sprent and Miller, 1972). However, there is recent evidence that long lived T cells might have a longer average life span than long lived B cells (Sprent and Basten, 1973; Sprent, 1974).

3.2 Surface characteristics

In recent years, the lymphocyte surface has been the subject of much careful investigation. Important differences between the surfaces of T and B cells have been demonstrated. Such surface differences have proved invaluable in fractionating, purifying and characterising lymphocytes, and have contributed much to the rapid advance of experimental immunology over the last decade.

Surface antigens

Probably the most important surface characteristic to be described has been the theta alloantigen demonstrated by Reif and

Allen (1963) on mouse thymocytes and later demonstrated by Raff (1969) on peripheral T cells. It is defined by alloantibody made in one strain of mouse against thymocytes of another and, being absent from B lymphocytes, provides a specific and convenient marker for thymus derived cells. B cells on the other hand, have been shown to possess easily demonstrable surface immunoglobulin, and a heteroantigen MBLA (mouse specific B lymphocyte antigen) which can be defined by heteroantibody made in rabbits against mouse B cells (Raff et al., 1971).

Receptors

In addition to antigenic differences, specific receptors have also been identified on the surface of lymphocytes. B cells have been shown to possess complement receptors for binding to antibody - antigen - complement complexes by Nussenzweig and colleagues (Bianco et al., 1970), and also receptors for the Fc part of complexed immunoglobulin (Fc receptors) by Basten et al. (1972 a & b). However, not all B cells carry complement receptors, for large numbers of cells lacking such receptors have been demonstrated in the peripheral lymphoid tissues of nude mice and they also appear to be absent from antibody forming cells (Dukor, cited by Greaves et al., 1973).

Fc receptors are thought to occur on all mouse B cells, and although not present on resting T cells, they have been demonstrated on activated T cells (Yoshida and Andersson, 1972). They are also present on cells of the monocyte macrophage series (Van Furth, 1974).

3.3 Responsiveness to mitogens

T and B lymphocytes can also be distinguished by their ability to respond in vitro to a range of mitogens such as the phytomitogens, bacterial products, or antibodies to lymphocyte surface antigens. While plant lectins such as Phytohaemagglutinin (PHA), Concanavalin A (Con A) and Lentil have been shown to stimulate T cells, other mitogens such as Bacterial lipopolysaccharide (LPS) act selectively on B cells, while still others such as Pokeweed mitogen (PWM) have the ability to stimulate both T and B cells. The responses of T and B lymphocytes to these polyclonal mitogens have provided convenient methods for assaying the functional state of the lymphocyte pool in experimental and clinical situations. However, recent evidence suggests that responses of lymphocytes to these mitogens may not be as uniform as was originally supposed. For instance, Stobo and Paul (1973) have evidence for differences between Con A and PHA responsive T cells in mice; Con A can activate a sub population of T cells which is unresponsive to PHA.

3.4 Sensitivity to immunosuppressive agents

Antilymphocyte serum (ALS)

ALS is a powerful immunosuppressive agent which exerts its effects by preferential depletion of the recirculating pool of T cells (Lance, 1970). Evidence for its effect on this cell population has come from observations on ALS treated mice which have been shown to possess a markedly reduced ability to mount T cell dependent responses (cell mediated functions such as delayed hypersensitivity,

graft rejection and thymus dependent humoral responses) (Lance, 1970), depleted thymus dependent areas (Taub and Lance, 1968), few theta positive and PHA responsive cells or thoracic duct lymphocytes (Schlesinger and Yron, 1969; Tyler et al., 1968).

Although T dependent humoral responses are impaired, responses to thymus independent antigens are unaffected, B cells appearing to be insensitive to ALS. This sparing of B cells is thought to be due to the inability of ALS to penetrate lymphoid tissue (Denman and Frenkle, 1968); most B cells being non-recirculating, remaining within lymphoid tissue.

The insensitivity of secondary humoral responses to ALS is also thought to be due to the presence of some non-recirculating T cells (Lance, 1970).

Drugs

The effects of a range of drugs on T and B cell responses have been studied (reviewed by Gabrielson and Good, 1967). While some of these drugs have been shown to exert preferential effects on either T or B cell responses, others show little or no specificity. The majority of them are cytotoxic and appear to interfere with cell proliferation. The effect of cyclophosphamide, on mice and guinea pigs has been investigated in some detail (Turk and Poulter, 1972) and appears to deplete B cells mainly, there being a sparing of T cells and T dependent areas in treated animals. However, the more recent work of Jokipii and Jokipii (1973) who observed effects on cell mediated responses as well, have cast doubts on the specificity of this

drug.

Azathioprine (Im uran) on the other hand, appears to have a greater effect on T cells; in mice, T-rosette forming cells are more sensitive to its effects than B-rosette forming cells (Bach, 1973).

Corticosteroids

Corticosteroids are immunosuppressive because of their anti-inflammatory and lympholytic properties, and in mice different lymphocyte populations show varying susceptibilities to steroids. In peripheral lymphoid tissues, they act mainly on B cells; helper T cells and GVH responsive cells being unaffected (Cohen and Claman, 1971). Antibody forming cells are also insensitive to the effects of steroids, hence humoral responses, once established, are steroid resistant.

The effects of steroids on thymic cortical cells are well established (Warner, 1964). They are also known to inhibit PHA responsive cells in the mouse and may also interfere with T cell recirculation (Levine and Claman, 1970; Cohen, 1972).

4. CELL INTERACTIONS AND IMMUNE RESPONSES

4.1 General comments

So far the nature and properties of immunocompetent cells have been outlined. Some functional aspects of responses mediated by these cells will now be discussed.

When an immunogen comes in contact with and combines

with its corresponding receptor on an immunocompetent cell, one of many things can happen; it can trigger off a response, induce a state of non responsiveness or paralysis; alternatively it may not be influenced by the encounter. Whether or not a response is initiated (i.e. whether an immunocompetent cell is switched on or off), and the nature and kinetics of a response if it is triggered depend on a number of factors. The nature and concentration of the immunogen, its immunogenicity and mode of presentation, the type, functional status and previous experience of the immunocompetent cell are some of the criteria involved.

Both T and B cells are able to respond to antigens, but while a number of antigens trigger off responses in both T and B cell pools, others tend to preferentially activate only one cell type. Antigens which initiate responses in the T cell pool are termed thymus dependent antigens, while others which provoke responses exclusively in the B cell pool are termed thymus independent antigens. Complex antigens are likely to possess both thymus dependent and independent components. The main features and significance of such thymus dependent and independent responses will be considered.

4.2 Thymus dependent responses

Thymus dependent responses are those which depend on the T cell pool for their expression. Whether an immune response is thymus dependent or not, can be ascertained by comparing the ability of normal and T cell depleted animals or cell populations to

carry out that particular response. T cell dependent immune reactions include cell mediated responses (such as delayed hypersensitivity, graft rejection and GVH reactions) in which T cells act alone or synergistically with macrophages, monocytes, etc. (no antibody is involved in such reactions); and thymus dependent humoral responses in which T cells co-operate with B cells in the production of humoral antibodies, the T cells serving a "helper" function. The essential features of these different types of T cell dependent responses will be described.

Cytotoxicity mediated by specifically sensitized T cells

When an antigen is a normal or abnormal constituent of a cell membrane, antigen stimulated T cells differentiate into specific cytotoxic T lymphocytes, which are able to destroy directly target cells carrying the sensitizing antigens (reviewed by Cerottini and Brunner, 1974). This phenomenon has been studied extensively in allograft and tumour systems (Perlmann and Holm, 1969). With appropriate methods such as measuring the release of ^{51}Cr from labelled target cells, it is possible to quantitate the activity of such cytotoxic cells and hence study their role in graft rejection, tumour immunity and immunity to infectious agents.

The mechanism of T cell mediated cytotoxicity has not been defined in molecular terms. Following specific contact between a cytotoxic T lymphocyte and the relevant target cell, irreversible changes in the permeability of the target cell membrane are thought to occur rapidly. One cytotoxic lymphocyte can lyse several target

cells. The reaction is independent of complement, and is inhibited by antibodies directed against the relevant target cell antigens.

Cytotoxicity mediated by macrophages

When immune T cells are confronted with specific antigen they can also elaborate various non antigen specific factors or "lymphokines" (Dumonde et al., 1969) such as migration inhibition factor (MIF), chemotactic factor, mitogenic factor, etc., which are primarily concerned with "activating" macrophages. As a consequence, macrophages acquire an increased non specific microbicidal capacity and may exert a non specific cytotoxicity.

In vitro studies have shown that normal macrophages may also be made specifically cytotoxic by contact with immune lymphoid cells or cell free supernatants from cultures of immune lymphoid cells incubated with antigen. The cell free supernatants appear to contain a specific arming factor (SMAF) elaborated by immune cells which are cytophilic for, and confer specific activity on, macrophages (Alexander et al., 1972).

Antibody dependent cell mediated cytotoxicity

Target cells coated with IgG antibody may be specifically lysed in vitro in the presence of normal lymphoid cells carrying surface receptors directed against the Fc portion of the IgG molecule (reviewed by MacLennan, 1973; Forman and Moeller, 1973). The effector cells have not been definitely characterized but a "third population" of lymphoid cells (non T; non B; non adherent and non phagocytic) have been implicated and shown to lyse antibody coated

chicken RBC, and tissue culture cells in vitro. Although this form of cytotoxicity is mediated by a non T cell, IgG production and hence the overall response is thymus dependent. This mechanism is discussed further in Chapter 5.

Thymus dependent humoral antibody responses

Although T cells do not secrete antibody like B cells, it is now established that they play an important role in helping B cells to make antibody to a wide range of antigens - T cells acting as "helper" cells. The demonstration of such T-B cell synergy was a significant advance in immunology since it explained the impaired humoral responses in T cell deficient animals and in congenitally hypothymic children.

Evidence for such T-B cell co-operation came from the work of Claman et al. (1966) who showed that irradiated mice given both thymus and bone marrow cells made a greater antibody response to SRBC (sheep red blood cells) than recipients of only thymocytes or bone marrow cells. Subsequently, Davies et al. (1967) showed that T cells could not make antibody, while Miller and Mitchell (1968) provided further evidence that the antibody forming cells were in fact derived from the bone marrow and were not T cells.

Although it is now established beyond any doubt that T-B cell co-operation occurs, the mechanism of such co-operation remains controversial. The studies of Mitchison et al. (1970) with chemically defined antigens have shown that T-B cell co-operation involves T cells responding to one antigenic determinant on an

immunogen, and helping B cells to recognize a different determinant on the same immunogen. This gave rise to the concept of an "antigen bridge" and although it now seems that such a bridge between T and B cell receptors is essential for co-operation to occur, it is uncertain whether the bridging occurs between T and B cells themselves, or between released T cell receptors and B cells. Such receptors might be taken up on the surface of macrophages which then bridge with B cells. Variations of this model have been proposed by Bretscher and Cohn (1970) and by Lachmann (1971). However, Feldman et al. (1973) have presented striking evidence that in some in vitro responses co-operation may involve the release by T cells of antigen specific IgM like factors complexed with antigen, which are subsequently taken up by macrophages. The precise functional significance of such an "antigen bridge" is uncertain. It could serve to present antigen to B cells in a particularly immunogenic form and might also help to bring B and T cells together so that short range factors released by T cells might operate.

The amount of T cell help required for the production of T dependent antibody varies considerably depending on the class of antibody involved. For instance, IgG responses are more T cell dependent than IgM responses. Nude mice injected with SRBC contain direct IgM but few indirect IgG plaque forming cells (PFC) in the spleen, and produce haemolysins but not haemagglutinins (Kindred, 1971; Pantelouris and Flisch, 1972; Wortis, 1971). In T cell deprived mice injected with SRBC IgG, PFC are reduced

rather than IgM PFC (Taylor and Wortis, 1968) and the IgG_1 response to KLH (Keyhole limpet haemocyanin) and BSA (Bovine serum albumin) is reduced more than other sub-classes in thymectomized mice (Torrigiani, 1972). In the mouse anti-SRBC response the amount of T cell help required seems to decrease in the order of IgG_1, IgG_{2a}, IgG_{2b} and IgM. IgA and IgE antibody responses also appear to be T cell dependent. Further, although IgG antibody production is usually T cell dependent, IgG serum immunoglobulin levels are within the normal range in neonatally thymectomized mice (Arnason et al., 1964; Fahey et al., 1965; Humphrey et al., 1964).

4.3 Thymus independent responses

Certain immunological responses can occur in the absence of thymus influence - thymus independent responses. Generally, a response is considered T independent if a T cell depleted animal does not differ significantly from an appropriate control in its response to the immunogen.

A number of antigens are known to elicit T independent responses - pneumococcal polysaccharide (Howard et al., 1971), bacterial lipopolysaccharide, polyvinyl pyrrolidone (Andersson and Blomgren, 1971), and polymerized flagellin (Armstrong et al., 1969). These antigens are characterized by the fact that they are large polymeric molecules with repeating identical determinants, are poorly catabolised, and persist for long periods in vivo (Felton, 1949). The response to such antigens often tends to be macrophage as well

as T cell independent, and is almost exclusively an IgM antibody response. T independent antigens tend to induce poor immunological memory.

4.4 Significance of thymus dependence

The exact biological significance of thymus independent IgM responses in animals possessing a full complement of T cells and the ability to produce a wide range of immunoglobulins is obscure. But such responses could be important in protection against bacterial antigens since these possess repeating determinants and are good examples of T independent antigens. Protection against such antigens is thought to depend on the rapid production of antibody with the capacity to neutralise their toxic effects, and T independent IgM responses probably fulfil this role.

However, responses to other micro-organisms, intra- and extra-cellular parasites and tumours depend directly or indirectly on the T cell pool, despite the fact that some of these antigens such as the micro-organisms probably carry repeating determinants on their surface. Basten and Howard (1973) have argued that these immunogens probably "see" both T and B cells, but the hosts have opted to develop T dependent immune processes because responses dependent on B cells alone might be limited in some respects, and hence could prove a distinct disadvantage in terms of survival of the host. For instance, the inability of B cells to develop memory or be rapidly mobilised might be a serious disadvantage to the host, whereas if antigen reacted with T cells in

the recirculating pool it would bring into operation a much wider range of responses. Interaction with T cells would permit the development of a full range of humoral responses, mobilization of other effector cells due to the release of factors by sensitized T cells, the initiation of inflammatory responses, kinin release, complement and coagulation cascades and phagocytosis (Good et al., 1971). Hence interaction with T cells permits diversification of the immune response and activation of a much wider range of host defences.

5. MODULATION OF IMMUNE RESPONSES

Not only is the vertebrate immune system capable of effecting a wide range of immune responses, but its immunocompetent cells have also developed the potential to carry out a complex range of homeostatic mechanisms by means of which they regulate each others functions. Both T and B cells are now known to be capable of suppressing and enhancing immune responses.

5.1 Suppression of immune responses

Passively administered antibody has been known to exert suppressive effects by "feedback inhibition" (Uhr and Moeller, 1968). Such antibody mediated suppression is immunogen specific and could be due either to the antibody masking determinants on the immunogen or preventing the immunogen from coming in contact with B cells. Evidence for the latter possibility comes from the work of Sinclair (1969) who found that removal of the Fc fragment reduces the

suppressive effect of such antibody.

Antibody can also suppress T cell responses. Passively administered antibody enhances tumour growth in vivo, while antibody or antigen-antibody complexes in the serum of tumour bearing animals prevents the animals T cells from killing the tumour cells (reviewed by Hellström and Hellström, 1974). The mechanism of this suppression of T cell function is controversial but antibody could react with antigen thus protecting it from T cell attack or the antibody could cover antigenic determinants on the tumour hence preventing it from eliciting an effective response in the T cell pool. There is also some evidence that B cells exert control on other T cell responses such as delayed hypersensitivity. B cell depletion of guinea pigs with cyclophosphamide results in enhanced T cell responses such as the Jones-Mote form of delayed hypersensitivity (Turk and Poulter, 1972).

The ability of T cells to function as "helper" cells has been discussed. In recent years a considerable amount of evidence has accumulated suggesting that T cells may also suppress immune responses and hence function as "suppressor" cells (reviewed by Droege, 1973a; Gershon, 1974). In view of this functional duality possessed by T cells, the term "regulator cell" (Katz and Benacerraf, 1972) has been suggested as being more appropriate. Both specific and non specific suppression has been attributed to T cells.

Specific suppression by T cells

The first report of antigen specific suppression came from Horiuchi and Waksman (1968) who induced whole animal tolerance in rats, by the intra-thymic injection of BGG (Bovine Gamma Globulin). Thymocytes from such BGG inoculated rats were later shown to be capable of transferring tolerance to syngeneic recipients (Ha and Waksman, 1973).

A number of experiments in which adult thymectomized and ALS treated mice have given enhanced responses to T independent antigens such as pneumococcal polysaccharide (Baker et al., 1970; Kerbel and Eidinger, 1971) have also been interpreted on the basis that in normal animals these antigens instead of eliciting T cell help cause T cells to produce suppressor factors which are specific. Droege (1971 and 1973b) has also characterized a bursa dependent population of "suppressor thymocytes", which have suppressive effects when transferred into normal chickens and which can transfer specific unresponsiveness if injected with antigen.

Non specific suppression by T cells

It has also been claimed that antigenic competition (Alder, 1964) is due to T cells directly or indirectly causing the production of suppressive factors (see Gershon, 1974). Although alternative explanations have been put forward to explain this phenomenon (reviewed by Pross and Eidinger, 1974), Gershon (1974) has marshalled a considerable amount of evidence supporting the idea that suppressive factors may be involved. These include the

observation that the phenomenon is often reversible in that cells undergoing competition respond normally when removed from the local environment (Waterston, 1970), and the demonstration by Veit and Michael (1972) of an increase in a factor which could directly and non specifically inhibit T cell responses in the serum of mice showing competition.

Although the literature has been flooded in recent months with reports of specific and non specific suppression mediated by T cells, the identity and precise mode of action of suppressor T cells is still obscure. Droege however has speculated on the possibility that these cells might reside in the thymic cortex (Droege, 1973a).

5.2 Enhancement of immune responses

Antibody has been shown in certain situations to enhance humoral responses to the corresponding antigen (e.g. Henry and Jerne, 1968). Enhancement of both IgM and IgG responses has been demonstrated. While the work of Dennert (1971) suggested that the enhancing effect of IgM anti SRBC antibody was related to its capacity to concentrate antigen in the spleen, the more recent demonstration by Schierman and Mcbride (1972), that in neonatally thymectomized chickens antibody can suppress but not enhance responses to allogeneic RBC, has implicated T cells in the phenomenon of antibody induced enhancement. Although there have been suggestions that antibody might also enhance "pure" T cell responses e.g. acceleration of graft rejection (Baldamus et al., 1973) the

evidence is limited and not very convincing.

SECTION II. THE IMMUNE RESPONSE TO INFECTIOUS AGENTS

1. GENERAL COMMENTS

The range of immune responses available to a vertebrate host has been outlined. The manner in which the host utilises these responses to defend itself against infectious agents will now be considered.

As the properties of pathogenic microbes are extremely varied, the host has evolved an equally wide repertoire of resistance mechanisms which it can use in self defence. For instance, the components of immunity involved in resisting viral infections include the resistance of individual cells to infection, local factors such as temperature or acidity, the formation of interferon, the resistance of macrophages, and specific humoral and cell mediated responses (Allison, 1972).

Infectious agents in turn have developed a series of subtle mechanisms by means of which they attempt to outwit the host. This is well exemplified by some protozoal and metazoal parasites which are able to evade specific immune responses and persist in immunised hosts by modulating their surface antigens (Trypanosomes and malaria parasites), acquiring host antigen on their surface (Schistosomes), or persisting in immunological inaccessible sites (Trypanosoma musculi).

However, it is not intended in this section of the review to catalogue the numerous interactions between hosts and different infectious agents in any great detail; rather, it is an attempt with the use of a few selected examples, to highlight the diversity of specific immune mechanisms used to combat infectious agents. Innate resistance mechanisms will not be discussed. For the sake of convenience, humoral and cell mediated responses are discussed separately, but it can hardly be over-emphasized that there is considerable interaction between these two efferent limbs of the immune response which often act together to control infections.

2. ANTIBODY MEDIATED RESPONSES

Serum antibodies are known to be important in controlling a number of bacterial, viral and protozoal infections (reviewed by Allison, 1974; Mauel and Behin, 1974). The importance of antibody is usually dependent on whether the infectious agent is an intracellular or extracellular pathogen, and on its mode of replication and dissemination. If the infectious agent concerned is an intracellular parasite which can multiply and spread without recourse to an extracellular environment, antibody would have no access to it, and by itself would be quite ineffective in controlling such an infection.

Protective antibodies can act on infectious agents in a number of different ways. In bacterial infections antibodies can be purely antitoxic, in which case they are concerned with the prevention of tissue damage that favours growth of the organism. Alternatively, antibodies to cell wall antigens can be lytic, effecting bacteriolysis

in the presence of complement; both IgM and IgG can serve as lytic antibodies. In other situations the antibody can collaborate with phagocytic cells and act as opsonizing or cytophilic (Boyden, 1974) antibody.

In a number of viral infections, antibodies act by neutralizing a virus either at the point of entry - such as a mucous membrane, or in the blood, and hence prevent its dissemination. There have been recent reports (Shore et al., 1974; Rager-Zismann and Bloom, 1974) that antibody dependent cytotoxicity might also be an important mechanism in controlling the spread of viral infections. The prediction of Allison (1972) that this mechanism would operate in viral infections has been confirmed by the demonstration that antibody acting synergistically with non immune effector cells can destroy herpes simplex virus (HSV) infected target cells.

The role of antibody in infections has been analysed to a large extent by the passive transfer of serum antibodies from immune to non immune recipients. A recent modification of this experimental approach has been to immunosuppress the recipients with cyclophosphamide, and then determine whether serum antibodies can reverse the increased susceptibility of these animals. Using the latter approach Nathanson and Cole (1970) and Allison and coworkers (reviewed by Allison, 1972) have established an important role for serum antibodies in a number of viral infections.

3. CELL MEDIATED RESPONSES

Pathogens which are intracellular, particularly those which can survive and multiply within mononuclear phagocytes and are hence largely sheltered from serum antibodies, tend to elicit cell mediated immune (CMI) responses. The manifestations of CMI used to resist infectious agents include direct killing by sensitized T cells and mechanisms in which there is collaboration between T cells and macrophages.

Direct T cell killing appears to be limited to viral infections, in which cells containing growing virus or expressing viral antigens on their surface are recognized and destroyed by specifically sensitized lymphocytes (Speel et al., 1968); the mechanism is particularly relevant to cells containing slowly multiplying or oncogenic viruses. This mechanism has not been shown to operate in bacterial or protozoal infections. Although Bray and Bryceson (1968) and Bryceson et al. (1970) claimed there was direct killing of Leishmania enriettii infected guinea pig macrophages by sensitized lymphocytes, the process has now been shown to be non specific (Mauel and Behin, 1974).

In the majority of infections in which cell mediated responses are an integral part of the protective response there appears to be synergism between sensitized T cells and other non specific inflammatory cells such as macrophages. Specifically sensitized T cells usually recognize antigens in foci of infection and trigger off a response resulting in an influx of inflammatory cells to the site of infection.

The interaction between T cell and macrophage can include attraction, arrest, and activation of the macrophage and is thought to be mediated by a range of lymphokines (Dumonde et al., 1969) such as migration inhibitory factor (MIF), macrophage attraction factor (MAF), etc.. The importance of these different phases of macrophage activity varies in different infections, while in some attraction is of prime importance, in others the key event is activation.

Two infections in which these phenomena have been examined in considerable detail are Listeria monocytogenes (a bacterial infection) and ectromelia virus infection (mouse pox) (reviewed by Blanden, 1974). Macrophages from Listeria infected mice possess enhanced non-specific microbicidal activity produced by specific immunological processes. Resistance to infection develops in parallel with macrophage activation and the development of delayed hypersensitivity to Listeria antigens. Immunity and delayed hypersensitivity are transferable with immune lymphocytes but not with serum. Treatment of these cells with anti-theta antiserum abolishes their effect, implying that the effective cells are T cells (Lane and Unanue, 1972; Youdim et al., 1973).

In ectromelia infections in the mouse, cell mediated responses are crucial for the development of immunity (Blanden, 1974). Blanden (1970, 1971a & b) has analysed the host response to this infection in great detail, and has shown that T cells are essential for initiating recovery from infection. Treatment of infected mice with ALS abrogates the protective response but anti-viral antibody titres and

interferon levels are unaffected, suggesting that these factors do not contribute significantly to the protective response. Specifically sensitized T cells appear to recruit inflammatory cells to foci of infection and phagocytes in this infiltrate engulf the virus. Macrophage activation occurs but is thought to be of secondary importance.

In certain situations, accumulation and activation of macrophages are ineffective in eliminating the infectious agent, e.g. Mycobacterium tuberculosis. In this case the influx of mononuclear phagocytes continues and granuloma formation results (WHO, 1973).

Cell mediated phenomena are also known to be important in a number of other infections where the parasite can survive and multiply within macrophages, e.g. Leishmania, Toxoplasma and Trypanosoma cruzi. Macrophage activation occurs in toxoplasmosis (Hirsch et al., 1974) and in some host-parasite combinations of Leishmania (Mauel and Behin, 1974) but the immune response to these infections probably involves other components as well which are still incompletely understood.

4. IMMUNOLOGICAL CONSEQUENCES OF INFECTION

An important aspect of many infections is that they result in a state of depressed immunological responsiveness. This phenomenon was observed as early as 1908 by Von Pirquet who noticed that tuberculin reactions became negative during an attack

of measles. Such depressed immunological reactivity as a consequence of infection has now been demonstrated in a wide range of viral, bacterial and protozoal infections.

Viral infections for instance have been shown to depress humoral immunity (e.g. Aleutian mink disease, Marek's disease, Newcastle disease, and the murine leukaemia viruses) or cell mediated immunity (e.g. lactic dehydrogenase virus, influenza, chicken pox, polio), and various mechanisms including the infection and destruction of lymphoid cells have been implicated in the observed effects.

Lactic dehydrogenase virus which depresses cell mediated immunity has been shown to cause a rapid depletion of the thymic cortex and thymus dependent areas of peripheral lymphoid tissue (Snodgrass and Hanna, 1970; Snodgrass et al., 1972). Active destruction of lymphocytes in thymus dependent areas has also been observed in lymphocytic choriomeningitis (LCM) (Hanaoka et al., 1969), an infection which depresses both humoral and cell mediated responses (WHO, 1973). Newcastle disease in chickens and Rinderpest in cattle also markedly depress CMI responses and this has been attributed to the direct cytotoxic effects of virus on lymphoid cells (WHO, 1973). Lymphocytes from patients with Rubella show depressed PHA responses and if normal lymphocytes are infected in vitro inhibition of PHA responsiveness occurs (Bellanti et al., 1965; Montgomery et al., 1967; Olson et al., 1967). Direct inoculation of a number of other viruses - polio, mumps and influenza have

essentially similar effects (Willems et al., 1969), suggesting that in these infections the virus directly or indirectly affects T cells.

The immunodepressive effects of the murine leukaemia viruses (Friend-Rauscher and Gross-Moloney groups) have also been extensively investigated because of their similarity to the human disease, and suppression of humoral responses to a wide range of antigens - SRBC, T_2 phage, bovine serum albumin (BSA) - has been reported (Siegel and Morton, 1966; Salaman and Wedderburn, 1966; Ceglowski and Friedmann, 1968; Peterson et al., 1963). The Rauscher leukaemia viruses (RLV) have also been shown to retard the development of autoimmune haemolytic anaemia and delay the appearance of antinuclear antibodies in NZB mice (Siegel and Morton, 1970; Siegel et al., 1972). Lymphoid cells from mice infected with RLV have also been found to give reduced responses to PHA (Hayry et al., 1970).

Lepromatous leprosy is probably the best studied example of a bacterial infection which causes depressed immunological activity. Patients with lepromatous leprosy, the severe form of this spectral disease, show an anergy to antigens of Mycobacterium leprae in the lepromin skin test, while delayed hypersensitivity to this antigen is pronounced amongst patients with the milder tuberculoid form and in normal controls. Also patients with leprosy produce high levels of circulating antibody to leprosy bacilli (e.g. Jha et al., 1971) suggesting a disturbance of specific cell mediated immunity to antigens of the causative organism. However, there is considerable evidence favouring a generalised rather than a

specific suppression of CMI in lepromatous leprosy. For instance, most patients fail to become sensitised to 2-4 dinitrochlorobenzene (Waldorf et al., 1966), T dependent areas of peripheral lymphoid tissues are depleted of lymphocytes and packed with Mycobacterium leprae (Turk and Waters, 1968), there is a decrease in the number of T cells in the peripheral blood (Dwyer et al., 1973; Gajl-Paczalka et al., 1973; Wong et al., 1971), and increased survival of skin allografts (Han et al., 1971).

There is however no evidence of an increased incidence of other infections or tumours in patients with leprosy and responses to certain antigens such as KLH (keyhole limpet haemocyanin) are normal. It has therefore been argued that the depression of cellular immunity is partial rather than absolute, and that the deficiency which exists is probably secondary to a primary defect in the ability to eliminate M. leprae (Turk and Belehu, 1974). Non specific depression of cellular immunity has also been detected in individuals infected with Treponema pallidum. During primary and secondary syphilis depressed PHA responses have been observed and factors present in the serum during secondary syphilis depress the response of normal lymphocytes (Levene et al., 1969).

Most of the major protozoan infections - malaria, trypanosomiasis, leishmaniasis and toxoplasmosis - exert marked depressive effects on the immune system. Trypanosoma brucei infections reduce the antibody response to SRBC (Goodwin et al., 1972) and "protect" against allergic neuritis (an autoimmune disease

in which CMI is important) in rabbits (Allt et al., 1971). Toxoplasma gondii infections in mice depress the plaque forming cell response to SRBC, and cause marked depletion of the thymic cortex (Huldt et al., 1973), while infection of hamsters with Leishmania donovani impairs the humoral antibody response to ovalbumin (Clinton et al., 1969).

SECTION III. THE IMMUNE RESPONSE IN MALARIA

1. GENERAL COMMENTS

Of all infectious agents, malaria parasites probably constitute one of the most complex; a complexity which is largely a consequence of the life cycle of the organism. An infection induced in a mammalian host by the sporozoite (the infective form which develops in the mosquito) includes a phase of development in the parenchymal cells of the liver, followed by asexual and sexual cycles of development in the blood. The intracellular blood stages include a series of distinct developmental forms and an extracellular stage of development, the merozoite. These phases of maturation may be antigenically distinct, and may be present simultaneously or sequentially. Further, it is now established that some species of the parasite can modulate their antigens and so avoid destruction in a hostile environment.

Not surprisingly, unravelling the immune response to malarial parasites has proved difficult. The development of protective immunity has been shown to be thymus dependent in

rodents (e.g. Brown et al., 1969) and chickens (Jayawardena - unpublished results). Both humoral and cellular mechanisms have been implicated in the host response, but no clear understanding of how such mechanisms operate or the precise manner in which T cells influence these components of the immune response has emerged. The nature of the interaction between host and parasite is complicated by the ability of malaria to depress the immune response to certain other antigens, for this raises the possibility that the infection might damp down the response to the parasite itself. Evidence relating to the significance of these various components of the immune response and their relationship to the development of protective immunity, will be discussed.

2. MALARIAL ANTIGENS AND ANTIGENIC VARIATION

The nature and functional characteristics of malarial antigens are poorly defined. These antigens are not confined to the parasite but are detectable on the surface of parasitised cells, circulating in the plasma of infected individuals and in association with various tissues. Numerous attempts to study these antigens have met with only limited success (reviewed by Brown, 1969). Possibly the most significant contribution has been the recent work of Wilson et al. (1969, 1973) on the antigens of P. falciparum (reviewed by Wilson, 1974).

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susceptibility and differentiated between stable (S) antigens, labile (L) antigens and a group of intermediate susceptibility resistant (R) antigens. By growing P-falciparum in vitro, in the presence of radio-labelled amino acids, he demonstrated that while the heat labile (La^1 La^2 Lb) and the heat resistant (R) antigens are of parasite origin (they incorporate the label) the S antigens remain unlabelled and might therefore represent altered host erythrocyte components. These S antigens are found regularly in malarious (P. falciparum) plasma, are released in vitro only after red cell lysis and reinvasion have commenced, and are known to persist in the circulation, but provoke only a limited antibody response. They are also heterogenous in physical and serological properties.

The protective antigens of malaria parasites have not been identified, and the functional significance of the antigens characterised by Wilson is uncertain. The S antigens which are thought to be true soluble antigens might be important in the non specific immuno-depression observed. However, not all malaria parasites shed such antigens. While they appear to be a regular feature of P. falciparum, they have not been detected in P. malariae (Houba, 1974).

Superimposed on this inherent complexity of malarial antigens, is the parasites ability to change them continuously. Evidence for the occurrence of antigenically distinct populations in the asexual erythrocytic cycle has been found in P. knowlesi (Brown

and Brown, 1965), P. cynomolgi bastianelli (Voller and Rossan, 1969), and P. berghei (Cox, 1959; Wellde and Sadun, 1967). As early as 1938 Eaton, using the schizont agglutination test (the test involves interaction between antibodies and antigens on the surface of cells containing mature parasites), demonstrated a spectrum of variants in P. knowlesi infection. Brown and Brown (1965) showed that in chronic P. knowlesi infection each relapse was associated with a serologically distinct variant.

An important feature of some malarial infections is their chronicity which appears to be the result of the parasite's ability to survive and multiply in an immunised host. Antigenic modulation is thought to constitute the fundamental mechanism by which the parasite survives in such a hostile environment (Brown, 1971).

3. THE EFFECTS OF T CELL DEPLETION

The immune response to malaria was first shown to be thymus dependent by Brown et al. (1968). They studied P. berghei infections in normal and neonatally thymectomized rats and observed higher parasitemias and increased mortality in the thymectomized group. These results were confirmed by Stechschulte (1969a) who also compared antibody levels by the indirect haemagglutination and fluorescent antibody tests, and reported that there was no significant difference in antibody titres between normal and thymectomized groups.

Spira et al. (1970) studied the effects of rabbit anti thymocyte

serum (ATS) on the same host parasite combination and found that ATS virtually abolished the resistance of rats of different ages to a primary infection with P. berghei. ATS appeared to have a substantially greater suppressive effect than neonatal thymectomy on the host response. These workers also reported that humoral antibody production as judged by precipitin tests was unimpaired in the ATS treated group. On the basis of these results both Stechschulte (1969a) and Spira et al. (1970) claimed that cell mediated mechanisms were involved in the host response to P. berghei.

In a subsequent study Brown (1971) found that ATS treatment of rats chronically infected with P. berghei resulted in recrudescence of infection, and reduced the ability of cells from these rats to transfer immunity to normal syngeneic recipients.

Wright (Wright, 1968; Wright et al., 1971) examined the effects of neonatal thymectomy and ATS treatment on P. berghei infections in a different host - the golden hamster. His results were surprising as T cell depletion seemed to exert a protective effect. He found that while control hamsters survived 6 to 12 days, neonatally thymectomized hamsters lived for 19 to 25 days. Wright claimed that these hamsters died due to petechial haemorrhages in the brain caused by the production of an agglutinin. He suggested that T cell depletion impaired the production of this agglutinin, and thus extended the lifespan of these hamsters. Subsequently, Chapman and Hanson (1971) using what appeared to be the same system reported completely contradictory results. Comparing

the responses of normal and neonatally thymectomized hamsters, they observed faster development of the infection and earlier and higher mortality in the thymectomized animals.

Sheagren and Monaco (1969) examined the effects of rabbit anti-lymphocyte serum (ALS) on still another host parasite combination - a fulminant P. berghei infection in the mouse. They found that either ALS treatment by itself, or ALS treatment coupled with adult thymectomy caused a significant delay in the mortality of P. berghei infected mice. These treated mice consistently showed lower parasitaemias than the control groups. They reasoned that the immune response itself contributed in some way to the death of these animals; hence impairment of the response extended their survival time.

While much attention was focussed on the use of rodent models for studying immune mechanisms, the avian malarias were largely ignored despite obvious advantages inherent in the avian system. Longnecker et al. (1966) examined the effects of neonatal bursectomy and thymectomy on P. lophurae infections in chickens. While bursectomy impaired the host response thymectomy was reported to have had no effect.

A more extensive exploration of the system (Jayawardena, unpublished results) has shown that the development of immunity to P. gallinaceum in chickens is both bursa and thymus dependent. In these experiments chickens were bursectomized or thymectomized within 24 hours of hatch, and subjected to sublethal X-irradiation

(700 rads) the following day. They were infected with 1×10^4 P. gallinaceum parasitised RBC ten weeks later. Infection followed a fulminant course and proved fatal in bursectomized and thymectomized chickens, while in sham operated birds the infection was effectively controlled and eliminated. The effects of B cell depletion were also investigated in chickens chemically bursectomized with cyclophosphamide and similar results were obtained.

The effects of bursectomy on P. gallinaceum infections are a clear demonstration of the importance of humoral antibody in the development of a protective response. The similar effects of thymectomy on the host response may be suggestive of a thymus dependent humoral response being involved. Thymectomy in the chicken is known to impair the antibody response to certain antigens, and its differential effects on different immunoglobulin classes (Droege, personal communication) are similar to those reported in rodents. The possibility that a cell mediated component was also impaired in the thymectomized chickens cannot be ruled out.

This group of experiments in which methods of in vivo lymphocyte depletion were used to examine the role of T cells, produced some apparently conflicting observations and although a central role for T cells in the host response was implied, these experiments did not clarify with any certainty the precise manner in which these cells might mediate their effects. Selective restoration of the immune response in thymectomized animals might have clarified the relative importance of humoral versus cell

mediated components of resistance with greater precision.

4. CELL MEDIATED RESPONSES

The evidence for cell mediated mechanisms being involved in the protective response to malarial parasites is limited. Malarial antigen (P. knowlesi) in Freund's complete adjuvant (FCA) confers more complete protection than does the antigen in incomplete adjuvant (Phillips et al., 1970). The former procedure is thought to promote the development of specific cell mediated immunity and macrophage activation. FCA immunized monkeys show evidence of delayed type responses and lymphoid cells from these sensitized animals are responsive interms of increased DNA synthesis in vitro, to the sensitizing antigens. However, spleen cells from these monkeys have no direct cytotoxic effect on P. knowlesi infected erythrocytes (Phillips et al., 1970). As the authors have pointed out, the development of delayed hypersensitivity or increased DNA synthesis of spleen cells need not necessarily mean that these responses are involved in the mechanism of protection.

It has also been demonstrated that in the presence of specific antibody, macrophages from BCG infected mice take up the monkey malaria parasite P. knowlesi more effectively than normal macrophages (Brown, 1971), and this has been cited as evidence that macrophage activation may be important. However, there is no evidence that malaria parasites cause macrophage activation on a scale similar to that induced by infectious agents such as Listeria

(see section II of review).

It would be useful to determine whether BCG-activated mouse macrophages show similarly enhanced microbicidal capacities to rodent malaria parasites such as P. berghei in view of Mauel's observation (Mauel and Behin, 1974) that although macrophage activation is operative in the host response to Leishmania species to which the host is not susceptible, the mechanism is not involved in the response against parasites to which the host is susceptible.

5. HUMORAL RESPONSES

A substantial amount of experimental evidence suggesting a crucial role for humoral factors in the defence mechanism against malaria has accumulated.

Chronic malarial infections have been known to be a potent stimulus of immunoglobulin synthesis (Cohen, McGregor and Carrington, 1961). Increased levels of IgG, IgM, IgA and IgD have been detected in human malaria (McGregor et al., 1970) but the significance of the increase in IgA and IgD levels is uncertain since they show no correlation with the level of infection. Although much of the increase in immunoglobulins appears to be non specific, the existence of protective antibodies was demonstrated by Cohen et al. (1961). They found that protective antibodies to P. falciparum existed in the IgG fraction of immune human serum, an observation confirmed by Edozien et al. (1962) and Sadun et al. (1966). The

results of Cohen et al. (1961) also suggested that immune serum from which IgG had been extracted, but in which IgM remained, had little protective effect.

The protective effect of anti-malarial antibodies has been confirmed in studies on rodent systems. Resistance to P. berghei in rats can be transferred with serum; the protective activity of such serum was of short duration and was thought to be associated with 19S and 7S immunoglobulins (Diggs and Osier, 1969; Stechschulte et al., 1969). Lymphoid cells from P. berghei immune rats have also been shown to be capable of transferring immunity to normal syngeneic recipients (Phillips, 1970; Stechschulte, 1969b) and although this has been interpreted by some workers (Stechschulte, 1969) as being indicative of a cell mediated response, such cells might equally well have been concerned with antibody production. Evidence for the latter possibility is found in the work of Phillips and Jones (1972) who observed that recipients of lymphoid cells produced high levels of protective antibody and that maximum levels of such antibodies were reached at the time the infection was eliminated. Fractionation of the protective serum showed that maximum activity was associated with the G_1 fraction.

Cohen and coworkers (1969, 1970a, 1970b) using an in vitro approach have also presented strong evidence favouring a role for humoral antibodies. These workers studied the effects of serum from monkeys immune to P. knowlesi, on the growth of the parasite in vitro. (Parasite growth was assessed by the incorporation of

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^{3}H Leucine in to parasite protein.) They found that the anti serum had no effect on the growth of the intracellular parasite, but inhibited the cycle of growth which followed parasite division. The effect was complement independent and was associated with IgG and IgM antibodies. Bivalent peptic fragments were inhibitory but univalent Fab fragments were not effective (Cohen et al., 1969; Cohen and Butcher, 1970a; Cohen and Butcher, 1970b). These workers felt that the inhibitory antibodies were directed against merozoite antigens. More recently they (Butcher et al., 1973) have presented evidence suggesting that the susceptibility of a mammalian red blood cell to a malaria parasite is largely dependent on the presence of specific red cell receptors for the merozoite. If such a receptor does exist, then an antibody or other mechanism which blocked the binding of merozoite to red cell receptor would effectively prevent cell reinvasion and hence interrupt the life cycle of the organism.

Although these in vitro studies indicated that anti malarial antibody could be effective by itself, certain other observations have been interpreted to mean that the mode of action of this antibody might involve synergism with effector cells such as macrophages. The evidence for macrophage involvement has rested largely on the classical histological studies of Taliaferro and Mulligan (1937). They reported a considerable increase of the macrophage population particularly in the liver and spleen of infected monkeys, and observed that parasites in various stages of digestion were frequently present

within these macrophages.

In a more recent attempt to evaluate the role of macrophages critically, Crisswell et al. (1971) implanted millipore chambers containing parasites only, or parasites and macrophages in normal and P. berghei infected mice. They reported that destruction of parasites occurred when chambers were placed in infected mice, and the rate of destruction was significantly enhanced if the millipore chambers contained both parasites and normal macrophages. Alternatively, if the chambers contained a combination of parasites and macrophages from infected mice, and were implanted in normal mice, a similar increased rate of parasite destruction was observed. They suggested that humoral factors were acting synergistically with macrophages, and that macrophage activation may also have been involved. However, as the macrophage populations used were unfractionated peritoneal exudate cells, which in addition to macrophages would have contained other cell types, these experiments are open to criticism.

The experiments discussed so far established that anti malarial antibody was protective and suggested certain mechanisms by means of which such antibody might have exerted its effects. However, the cellular interactions involved in the production of these antibodies were not clearly defined. Brown (1971) using the results of his experiments on P. berghei infections in the rat, and his observations on antigenic variation in P. knowlesi, presented an integrated scheme to explain the cellular basis of antibody

production and its relationship to antigenic variation. He had observed that although an infected host could limit the proliferation of a relapse variant, it was fully virulent in a non immune host. He postulated that the protective antibody response was entirely variant specific, but that certain surface antigens common to all variants sensitized thymus derived cells; these acted as helper cells in antibody production and enabled the host to respond to new variants more rapidly.

6. IMMUNODEPRESSION

It is now established that malarial infections cause a state of reduced immunological responsiveness to certain antigens. The first observation relating to this phenomenon was made by McGregor and Barr (1962) who found that the immune response to tetanus toxoid was higher in children receiving malarial prophylaxis, than in unprotected children living in malarious areas. It had also been observed that Burkitt's lymphoma (Burkitt, 1959), a non leukaemic malignant lymphoma, occurred in definite geographical areas and a causal relationship between this lymphoma and holoendemic malaria was suggested (Dalldorf et al., 1964; Burkitt, 1969).

These initial observations which implied that malaria might exert a depressive effect on the immune system have now been supported by a considerable amount of epidemiological and experimental observations. The evidence comes from three main sources; the effects of malaria on malignant disease, on autoimmune disease,

and on the immune response to a wide range of test antigens.

6.1 Malaria and malignant disease

The precise relationship between malaria and Burkitt's lymphoma has not yet been resolved. It is possible that the increased incidence of this lymphoma which is associated with the Herpes like Epstein Barr virus (reviewed by Klein, 1971), might be due to malaria exerting a suppressive effect on one or more components of the immune system, thus permitting the full potential of this virus to be expressed. Alternatively, malaria might affect the lymphoid tissue in some way rendering it more susceptible to neoplastic transformation by the virus.

Wedderburn (1970) examined the possible relationship between malaria and malignant disease in an experimental system. She observed a much higher incidence of Moloney Lymphomagenic virus (MLV) induced lymphomas in mice infected with P. b. yoelii, than in those infected with the virus alone. She also reported that total virus neutralizing antibody and IgG antibody were absent in the doubly infected animals (Bomford and Wedderburn, 1973). More recently, she examined the effects of a chronic malarial infection (induced by infecting P. b. yoelii recovered mice with P. b. berghei) on MLV induced lymphomas, and reported a similar enhancement of lymphomagenesis in the malaria infected mice (Wedderburn, 1974). Earlier, Jerusalem (1968) found that mice repeatedly infected with P. berghei showed an increased incidence of malignant lymphomas or histological evidence of lymphomas.

6.2 Malaria and autoimmune disease

Epidemiological studies have indicated that autoimmune disease is relatively uncommon in humans continuously exposed to malaria and other parasitic infections. For instance, Greenwood (Greenwood, 1968; Greenwood et al., 1971) observed that rheumatoid arthritis and polyarthritis were rare or ran a benign course in Africans, although these diseases were common among American Negroes of African origin (Engel et al., 1966), and in Caucasians living in temperate climates. Greenwood suggested that malaria might be an important factor in the protection observed.

He tested out his hypothesis by infecting NZB mice with the malaria parasite P. b. yoelii, and observed that early infection of these mice was associated with a delay in the onset of Coomb's positivity and spontaneous haemolytic disease. Also, infection of (NZB x NZW)F_1 mice with the same parasite markedly suppressed the spontaneous development of autoimmune renal disease (Greenwood and Voller, 1970).

He also examined the effects of P. b. yoelii and P. b. berghei infections on experimental adjuvant arthritis in rats, and found that while infection of rats with P. b. yoelii one week before or at the same time as the injection of adjuvant resulted in the development of a milder form of arthritis than in controls, infection with the virulent P. b. berghei did not prevent the development of severe arthritis (Greenwood et al., 1970). The recent observations of Lennon and Byrd (1973) are perhaps relevant to these results. They found that

although cell mediated mechanisms were involved in this disease, neonatal thymectomy induced an undue proneness to it. They suggested that a decreased capacity to produce antibodies to thymus dependent antigens, might lead to the formation of complexes with antigen in excess, and that such complexes were responsible for mediating immune damage to tissues.

Although the ability of malarial infections to modify the course of autoimmune disease might be due to direct or indirect effects on immunocompetent cells, there is as yet no convincing evidence to support such a hypothesis. Greenwood and Voller (1970) in interpreting the results of their experiments, referred to the suggested viral aetiology of autoimmune disease, and indicated that their findings may be due to virus repressive rather than the immunosuppressive effects of these infections.

6.3 Malaria and altered responsiveness to test antigens

Subsequent to McGregor and Barr's (1962) demonstrations of altered responsiveness to tetanus toxoid in children exposed to malaria, the responses of humans and experimental animals infected with malaria to a wide range of antigens have been studied.

Greenwood (1974) assayed the immune competence of Nigerian children with acute P. falciparum malaria, and reported a diminished antibody response to Salmonella typhi O antigen and to tetanus toxoid. The response to the H antigen (which is essentially flagellin and is thought to be thymus independent) was normal. Cell mediated responses, as judged by skin testing with PPD (Purified

Protein Derivative), Candida and Streptococcal antigens were unimpaired. Also, there was no significant difference between patients and controls in the PHA responsiveness of their lymphocytes.

The phenomenon of immunodepression has also been investigated in some detail in the mouse host. P. b. yoelii, a non fatal infection, has been shown to depress the antibody and splenic plaque forming response to SRBC in Balb/C mice (Salaman et al., 1969; Greenwood et al., 1971a). The antibody responses to HGG (Heat aggregated Human Gamma Globulin) and tetanus toxoid are also greatly diminished, but the antibody response to certain other antigens such as KLH (Keyhole limpet haemocyanin) and bacteriophage are not significantly impaired (Greenwood et al., 1971; Voller et al., 1972; Barker, 1971).

Steward and Voller (1973) observed that although Simpson mice infected with P. b. yoelii developed normal levels of antibody to HSF (Human serum transferrin), the affinity of this antibody was lower than in normal mice.

Cell mediated responses such as contact sensitivity to picryl chloride or Oxazolone are not depressed and skin graft rejection times are not affected by P. b. yoelii infections (Greenwood et al., 1971).

Whitmore (1973) examining the effects of acute, fatal P. b. berghei infections on Swiss mice, observed depression of the plaque forming cell (PFC) response to SRBC and of delayed hypersensitivity responses to methylated human serum albumin (MHSA).

Sengers et al. (1971) reported a significant delay in skin graft rejection in the same host parasite system, but the infection had been prolonged by placing the animals on a para-amino benzoic acid free diet. It would seem that in acute infections (P. b. berghei in outbred Swiss mice) there is depression of both cell mediated and humoral responses, while in the milder P. b. yoelii infections only humoral responses to certain antigens are depressed.

Although the occurrence of immunodepression is now established beyond any doubt, the manner in which malarial infections induce this immunological lesion remains unexplained. Greenwood et al. (1971a) suggested that malaria might exert its suppressive effects by acting on B cells, macrophages or cell co-operation but produced no unequivocal evidence in favour of any of these possibilities. Macrophage activity does not appear to be depressed as judged by carbon clearance rates in infected mice. Increased clearance rates for carbon have been reported (Greenwood et al., 1971b). Hence it appears unlikely that reticulo-endothelial blockade contributes to the suppressive effects of these infections. Loose et al. (1971) have reported that macrophages from malaria infected mice are ineffective in detoxifying Endotoxin, hence it is possible that some aspects of macrophage function may be affected. Abnormalities in splenic function have also been observed. Greenwood et al. (1971b) found that HGG localised in the germinal centres of normal mice, but malaria infected mice failed to localise HGG in the germinal centres during the period in which immuno-

suppression was recorded.

Both the mechanism of resistance to infection, and the non specific immunodepression observed during infection, are probably dependent on complex effector and suppressor mechanisms. In studying the nature of these mechanisms, it seems important to bear in mind the complexity of the antigenic stimulus, which probably results in the generation of an equally complex array of responses in the host. Many of these responses may have no direct significance to the specific effector and suppressor mechanisms involved, but nevertheless make identification of the relevant responses a much more difficult task.

CHAPTER 2

THE MORPHOLOGY OF THE IMMUNE RESPONSE

Chapter Outline

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INTRODUCTION

The experiments reported in this chapter were concerned primarily with establishing whether the immune response to malaria in the mouse was thymus dependent, and in characterising further the nature of such dependency if it existed.

The main approach adopted was to study P. b. yoelii infections in normal CBA mice and in mice depleted of their thymus dependent lymphocyte population (T cell deprived), and to seek various descriptions of the immune response to the parasite in such normal and deprived mice. Particular emphasis was placed on studying the morphology of the immune reactions in the lymphoid system and in relating them to current concepts of thymus dependent and independent cell populations.

The immunological response of the CBA mouse to P. b. yoelii was then compared with its response to the closely related parasite P. b. berghei, infections of which always prove fatal in the normal CBA mouse, unlike P. b. yoelii infections which are relatively mild and non fatal in the same host. The host response to P. b. berghei appeared defective in some way, and an attempt was made to analyse the factors underlying the apparent failure of the immune system in these infections.

Finally, the effect of varying the host factor was investigated. Preliminary experiments had shown that the outbred T.O. mouse differed from the CBA mouse in its ability to respond

to a malarial infection. The immunological responses of this strain of mouse (to P. b. yoelii and P. b. berghei) were compared with the responses of the CBA mouse to obtain a better understanding of the possible importance of the host element in resistance mechanisms in malaria.

MATERIALS AND METHODS

1. THE PARASITES USED

1.1 Origin

The parasites used in this study were:

Plasmodium berghei yoelii (Landau and Killick-Kendrick, 1966). This parasite was first isolated from Thamnomys rutilans in the Central African Republic (Landau and Chaubaud, 1965). The 17X strain of this parasite which was stabilated (Lumsden and Hardy, 1965) as LUMP 605 was used.

Plasmodium berghei berghei (Vincke and Lips, 1948). This parasite was first described in Thamnomys surdaster by Vincke and Lips (1948). The Anka strain of this parasite, LUMP 58, which was used in the present investigation is derived from an isolation made in Zaire in 1965.

1.2 Cryopreservation

CBA mice which had been infected with a stabilate (LUMP 605) of the 17X strain of P. b. yoelii 5 days previously, were bled by cardiac puncture into heparinised phosphate buffered saline (PBS), to which glycerol was added to give a final concentration of 7%.

The blood was distributed with a Pasteur pipette into sterile capillary tubes, both ends of which were flame sealed, placed in a Methanol filled screw cap tube, and slowly frozen in a dry ice cabinet (Cunningham et al., 1963). This was designated LUMP 766 and was used to infect all mice which served as donors for the preparation of parasite inocula. All donors used for the preparation of P. b. berghei inocula were infected with stabilates from LUMP 58.

1.3 Preparation of infective inocula

All infective inocula of P. b. yoelii and P. b. berghei were prepared from donor mice which had been infected with the appropriate stabilate material 5 to 6 days previously. The parasitaemias of the donors were estimated from Giemsa stained thin blood films, and a total RBC cound was carried out using a Neubauer haemocytometer. The number of parasitised RBC/ml of blood was calculated. The donor mouse was then bled from the retro-orbital sinus into heparinized phosphate buffered saline (PBS), and the heparinised blood was diluted in chilled PBS to obtain the required inoculum. These procedures were carried out in an ice bath. All experimental animals were inoculated with the parasite inoculum intraperitoneally using a 1 ml syringe fitted with a 25G needle.

2. ESTIMATION OF PARASITAEMIAS

2.1 Thin blood films

Smears of tail blood were made on acetone cleaned glass

slides. The films were air dried, fixed in Methanol for 1 to 2 min and stained in a 17% solution of Giemsa (Revector brand, Hopkin and Williams) in buffered distilled water (pH 7.2) for 30 min. The slides were then washed momentarily in a gentle flow of tap water and stood upright to dry.

2.2 Counting of parasites

The stained blood films were examined on a Wild M20 microscope using an x100 (oil immersion) objective. The number of parasitised red blood cells (RBC) was counted in a minimum of 20 microscope fields and was expressed as a percentage of the total number of RBC scanned. Each field contained approximately 450 RBC.

3. MICE

3.1 Strains

CBA/Lac, CBA/H.T6T6, T.O. and Parkes mice were used.

3.2 Sources

CBA/Lac mice were obtained from the Chester Beatty Research Institute, London, and the Laboratory Animal Centre (Carshalton). All CBA/H.T6T6 mice were obtained from the Chester Beatty Research Institute. T.O. and Parkes mice were obtained from commercial breeders.

All mice were housed in mouse boxes in groups of 5 or 6, and were fed on standard mouse diet.

4. HAEMATOLOGICAL TECHNIQUES

4.1 Packed cell volume

Tail blood was allowed to run into heparinised micro-haematocrit capillaries (Hawksley) leaving at least 15mms unfilled. The tube was then sealed with Cristaseal and spun on a micro-haematocrit centrifuge (Hawksley) for 5 min. The packed cell volume was expressed as a percentage of the total volume occupied by the cells before centrifugation.

4.2 Reticulocyte counts

Blood films were made on slides previously coated with 0.3% Brilliant Cresyl Blue in 95% Ethyl alcohol. They were then incubated in a moist chamber for 5 to 10 min, air dried, fixed in Methanol and stained in Giemsa. 20 microscope fields were examined with an x 100 oil immersion objective and reticulocyte numbers were estimated as a percentage of the total number of RBC counted.

4.3 Total nucleated cell count

Blood was drawn up to the 0.5 mark in a haemocytometer pipette and diluted 1 : 20 with diluting fluid. (A 2% solution of Glacial Acetic acid in distilled water containing a few grains of Methyl Green). After thorough mixing, one cell of a Neubauer haemocytometer chamber was filled and counted. The number of nucleated cells in the four outer large squares was counted and multiplied by 50 for the number of cells/mm^3.

4.4 Differential cell count

Thin blood films were made on acetone cleaned slides. They were fixed immediately with 100% Methanol for 5 min, and stained in Giemsa for 15 to 20 min. At least 300 consecutive WBC were counted from each blood film. Neutrophil, lymphocyte and monocyte counts were computed from total and differential white cell counts.

5. HISTOLOGICAL TECHNIQUES

All tissues were fixed in Carnoy's fluid and processed according to standard histological methods. The staining techniques employed were:- Haematoxylin/Eosin, Methyl Green Pyronin, Gomori's Silver stain for reticulin and Perl's stain for Haemosiderin. These techniques were carried out according to the methods described in Carleton's Histological Technique, edited by Drury and Wallington (1967).

6. ESTIMATION OF BODY WEIGHTS AND ORGAN WEIGHTS

Mice were weighed on a balance (Berkel, Auto Scale) and the body weights determined. They were then killed by cervical dislocation, and the thymus, spleen, axillary and mesenteric lymph nodes and adrenals were removed and placed in a petri dish containing a filter paper moistened with sterile saline. The organs were weighed immediately on an Oertling balance, and the tissues were fixed in Carnoy's fluid for histology.

7. BLEEDING OF MICE

(From the retro-orbital plexus)

The mouse was removed from its box, and held firmly with the left hand. It was positioned on a work bench with its head turned partly onto its left side, right eye uppermost. Held in this immobile position the eye should be protruberant due to the engorgement of the venous plexus in the orbit. With the right hand a Pasteur pipette was now introduced into the medial canthus of the right eye directing it below the eye ball and towards the optic foramen. The pipette was rotated gently until it cut into the plexus. Once puncture had occurred the pipette was withdrawn very slightly and blood allowed to flow into it. After an adequate quantity of blood had entered the pipette, it was withdrawn from the eye and the blood discharged into a tube. After bleeding, the eye was washed with sterile saline. Using this method mice could be bled at weekly intervals and samples of up to 0.5 ml of blood could be collected. The technique has been described by Halpern and Pacaud (1951) and Sorg and Buckner (1964).

8. COLLECTION AND STORAGE OF SERUM

Blood was allowed to clot at room temperature, left overnight at $4^{o}C$ and the serum separated by centrifugation. The serum was inactivated at $56^{o}C$ for 30 min, and stored at $-70^{o}C$.

9. THE INDIRECT FLUORESCENT ANTIBODY TEST

9.1 Antisera

1. Lyophilised swine anti-mouse immunoglobulin conjugated to fluorescein isothiocyanate (FITC) (Nordic pharmaceuticals) was purchased from Fraburg Ltd. It was reconstituted with distilled water, diluted 1 : 2, and stored in aliquots of 100 µls at -20°C.

2. Goat anti-mouse IgG_1, IgG_2, IgM and IgA sera were purchased from Nutritional Biochemical Corp. Cleveland, Ohio, and kept frozen at -20°C. These antisera were conjugated to FITC according to the method outlined in Thompson's "Selected Histochemical and Histopathological Methods" (1966), and their fluorescein-protein ratios were determined according to the method of Holborow and Johnson (1967). These procedures were carried out by Dr P. Viens of this department. For details of procedures used and fluorescein-protein ratios see Viens (1972).

9.2 Fluorescent antibody titration

Preparation of slide antigen - CBA mice were infected with 1×10^5 P. b. yoelii or P. b. berghei parasitized RBC, and were bled into cold heparainised phosphate buffered saline (PBS) 10 days later. The blood was washed thrice in PBS (1000g at 4°C for 10 min). The cell pellet was resuspended in saline and thin films were made of the washed cells on acetone cleaned glass slides. The slides were air dried and stored at -70°C.

Procedure for carrying out test -

1. Slides were brought to room temperature in a desiccator and

then fixed in acetone for 1 to 2 min.

2. Each slide was next divided into 12 areas with a pentel pen.

3. Two-fold dilutions of the test serum were prepared and one dilution was applied to each area with a Pasteur pipette. All sera were diluted with PBS.

4. The slides were now placed in a humid perspex chamber and allowed to incubate for 30 min at room temperature.

5. Slides were then placed in coplin jars and washed three times in PBS (10 min x 3) on a Griffin flask shaker.

6. The slides were allowed to drain, excess PBS was removed, and the slides replaced in the humid chamber. They were flooded immediately with the appropriate conjugate containing 10% of a 1% solution of Evan's Blue in saline, and allowed to incubate for 30 min.

The fluorescein conjugated swine anti-mouse globulin was used at a final dilution of 1:40. The monospecific conjugates were used at dilutions of 1:20. Serial dilutions of these conjugates were assayed and these dilutions were found to maximise the sensitivity and specificity of the test.

7. The slides were washed once more in PBS (3 x 10 min), mounted in glycerine buffered to pH 8.0, and examined on a Zeiss photomicroscope illuminated with a high pressure mercury lamp HBO 200W and BG 38 and BG 12 exciter filters, and 50/44 barrier filters.

8. The degree of fluorescence was scored on a ++++ to + basis signifying a subjective scale of decreasing intensities of fluorescence,

and the last dilution showing a + was taken as the end point.

On all slides a (known) standard serum, a normal mouse serum and PBS were included as controls.

The antibody titre was expressed as the reciprocal of final dilution of the test serum which gave a positive staining reaction.

10. MEASUREMENT OF SERUM IMMUNOGLOBULIN LEVELS

The radial immunodiffusion technique of Mancini, Carbonara and Heremans (1965) was used to measure IgG_1, IgG_2 and IgM levels in mouse sera. The procedure was as follows:-

1. Preparation of agar.

To 100 ml of barbiturate buffer of pH 8.6 and ionic strength 0.1 (made by dissolving 9 gm of sodium diethyl barbiturate, 65 ml of 1/10 N HCl and 0.5 gm sodium azide as a preservative, in distilled water and adjusting the volume to 1 litre) was added 3 gm of Special Agar Noble (Difco). This suspension was placed on a boiling water bath and stirred until all the agar had dissolved. This stock solution of agar was stored at $4^{o}C$.

2. Preparation of agar-antiserum mixture.

To prepare the agar-antiserum mixture the required amount of solidified 3% agar gel was melted in a water bath and allowed to cool to $60^{o}C$. The antiserum or a suitable dilution of it in barbiturate buffer was brought to $55^{o}C$ after which it was mixed with the agar using a pipette which had been preheated to $60^{o}C$. The

agar-antiserum mixture was poured immediately into a perspex mould placed on a levelling tray, using the same preheated pipette. (The moulds used were those furnished by Hyland with their Human Immunodiffusion Plates.)

3. Preparation of the agar-antiserum plates.

A volume of 1.5 ml of the agar-antiserum was poured into each mould and this gave a gel 1 mm in thickness. Circular wells of 2 mm diameter were punched out of the gel and the agar removed by suction.

4. Application of antigen sample.

2 µl of antigen, i.e. test serum or standard serum, at suitable dilutions were placed in each well with a microsyringe. The plates were now placed in a humid chamber to prevent drying, and were left at room temperature for 48 hr.

5. Measurement of the size of precipitates.

The diameter of the ring shaped precipitates which formed round the wells was measured using a precision viewer (Hyland), and the areas within the rings were calculated. The area of the precipitate is directly proportional to the quantity of the immunoglobulin class in the test sample.

6. Quantitation of immunoglobulin levels.

The quantity of each immunoglobulin class in a particular serum sample was expressed as a percentage of the quantity found in a standard serum pool obtained from 12 to 15 week old outbred T.O. mice.

Four dilutions of this serum pool from outbred mice were assayed on plates made with each monospecific antiserum. The antisera used were goat anti-mouse IgG_1, IgG_2, and IgM and were obtained from Flow Laboratories. They were used at a dilution of 1:10. The standard lines obtained in this manner were used to calculate the percentage of the standard immunoglobulin level in a particular test serum. In addition to standard and experimental sera a single control serum was assayed on each plate set up.

11. PLASMA CORTICOSTERONE DETERMINATIONS

11.1 Collection and storage of plasma samples

Mice were bled from the retro-orbital sinus using heparin as an anticoagulant. Up to 0.5 ml of blood could be collected from each mouse by this method. Bleeding was completed within 30 sec or less, and Grigor (personal communication) has shown that there is no significant increase in plasma corticosterone levels due to the process of bleeding. The heparinised blood was placed on ice and the plasma separated immediately by centrifugation at 1000g for 15 min at $4^{o}C$ on a Mistral model 2L centrifuge. Plasma samples from individual mice were frozen in aliquots of 100 µl at $-70^{o}C$ until analysed. Mice were always bled between 9 a.m. and 10 a.m. to minimise changes in steroid levels due to diurnal variation.

11.2 Method of analysis

The method used to estimate plasma corticosterone levels was that used routinely by Dr K. M. Grigor of the Chester Beatty

Research Institute, London.

Reagents: The following reagents were required:

Chloroform

Sodium hydroxide 0.1N

Ethyl alcohol

Sulphuric acid

Glassware

All glassware including micropipettes used in these steroid determinations was washed first in Decon and then in tap water and distilled water before use.

Fluorescence reagent

The fluorescence reagent was prepared by adding 6.5 ml of concentrated sulphuric acid to 3.5 ml of ethyl alcohol in a flask which was cooled under tap water as the reaction was exothermic. The solution should remain colourless.

Corticosterone standards

A stock solution of corticosterone was prepared at a concentration of 10 µg/ml. This solution remains stable for months at 4°C. To prepare a series of standard solutions this stock solution was diluted to 1 µg/ml in ethyl alcohol, and volumes of this solution were pipetted out into a series of small pyrex tubes (0, 1, 5, 10, 20, 30 µl). These samples were then placed in a drying oven at 56°C for 30 min. The tubes were removed from the oven, allowed to cool, and 50 µl of distilled water were added to each tube. Hence the tubes contained 0, 1, 5, 10, 20 and 30 nanograms (ngm)/50 µl.

Extraction procedure

All samples were processed in duplicate. 50 µl of the test plasma were placed in a small glass tube. 25 µl of 0.1N NaOH were added to the plasma and they were mixed on a micro mixer. 200 µl of Chloroform were now added to each tube, the tubes were stoppered and mixed on a micromixer for a further 15 sec. The tubes were then spun on a microcentrifuge for 1 min at 100g. After spinning the aqueous layer was removed with a microaspirator fitted to a suction pump.

To 100 µl aliquots of the chloroform extract, 100 µl of the fluorescence reagent were added and they were mixed on a micro-mixer at 30 sec intervals. The tubes were spun immediately on a microcentrifuge at 100g for 2 min. The chloroform layer was then removed as completely as possible with a microaspirator, care being taken not to remove the fluorescence reagent.

Fluorimetry

The sulphuric acid layer which contained the steroid was pipetted into a fluorimeter cuvette, which was allowed to stand at room temperature. Exactly 45 min after the addition of the fluorescent reagent fluorescence was measured using a Aminco-Bowman fluorimeter. It was important to measure the fluorescence of each sample at the same interval of time after the addition of the fluorescence reagent so as to ensure that the degree of non-specific fluorescence remained relatively constant from estimation to estimation. To minimise such errors fluorimetry is best carried

out on batches of 6 to 8 plasma extracts, together with a blank and a set of standards.

Calculations

A series of corticosterone standards of known concentration and a reagent 'blank' (50 µl distilled water) were processed with each batch of plasma samples analysed. A standard curve of fluorescence vs. concentration of corticosterone in nanograms/50 µl was plotted on linear graph paper for each batch of samples. The corticosterone levels of the test samples were read off from this graph in ngm/50 µl and converted to µg/100 ml in which form they are expressed in the results reported in this chapter.

12. T-CELL DEPRIVATION

12.1 Method of thymectomy

(Plates 2.1a to 2.1f)

The method used for thymectomizing adult CBA mice, approximately 8 weeks of age, was that routinely used at the Department of Immunobiology, Chester Beatty Research Institute, London, and is based on that of Kaplan (1950) and Miller (1960).

Mice were anaesthetised with ether or Nembutal (Sodium pentobarbital) (0.1 ml of a 1 : 10 dilution was given intraperitoneally), and stretched out on a cork board with the ventral side uppermost. A wire loop attached to a rubber band was hooked over the upper incisors and pinned down, and a small cylindrical bolster was placed under the thorax. The skin was swabbed with 70% alcohol

to minimise infection.

A mid-line incision was made in the skin with scissors from 1 cm below the chin to approximately the level of the third rib. Using two pairs of fine blunt forceps the salivary glands were carefully separated, until the trachea and overlying fascia became visible. Next, a pair of fine scissors was inserted under the right clavicle and a cut was made slightly to one side of the sternum approximately to the level of the first rub. A similar cut was made on the left side, and this was followed by a transverse cut, so that a wedge of bone could be removed from the sternum. Next, a rectangular piece of fascia 3 mm wide and extending as far down the thoracic opening as possible, was removed so as to expose the thymus lying in the thoracic cavity. At this stage care was taken to prevent air from entering the thoracic cavity. Slight pressure was now applied below the diaphragm, so that the thymus lobes came to lie in the thoracic opening. Using a Pasteur pipette with a 2 to 3 mm diameter (i.e. just smaller than one thymus lobe) and connected to a negative pressure vacuum pump, each thymus lobe was carefully sucked out. After removal of the thymus lobes the incision was closed immediately to prevent air from entering. The two edges of the skin were held together with forceps and were joined with No. 7 Michel Clips or with 9 mm autoclips from an autoclip applier (Clay-Adams, Inc. N.Y.).

Mortality following the operation was approximately 10%. Sham operations were carried out in a similar manner but without

Method of Thymectomy

Plate 2.1a

Separation of salivary glands

Plate 2.1b

Section of claviculae

Method of Thymectomy

Plate 2.1a

Separation of salivary glands

Plate 2.1b

Section of claviculae

Plate 2.1c

Resection of sternal wedge

Plate 2.1d

Dissection of fascia

Plate 2.1c

Resection of sternal wedge

Plate 2.1d

Dissection of fascia

Plate 2.1e

Resection of fascia

Plate 2.1f

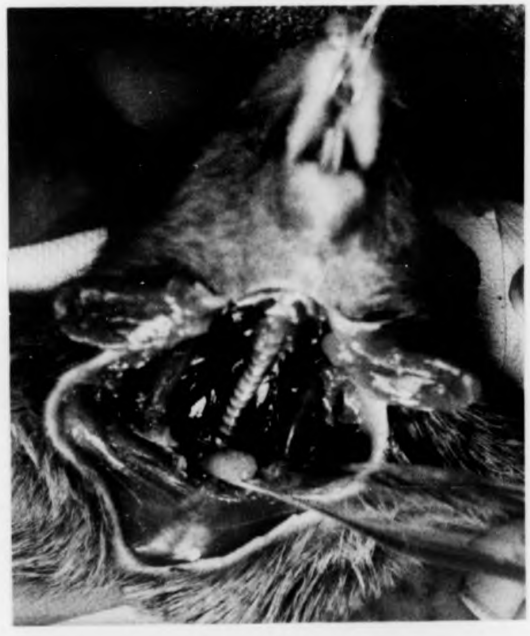
Aspiration of thymus lobes

Plate 2.1e

Resection of fascia

Plate 2.1f

Aspiration of thymus lobes



removal of the thymus lobes.

12.2 Irradiation

Mice were irradiated 10 days after thymectomy or sham thymectomy, with a Marconi Type TM 4574B irradiation unit made available by the Department of Physics in Medicine, Middlesex Hospital Medical School, London. For irradiation mice were placed in a 30 cm square perspex container, divided into 6 compartments. Each compartment could hold 5 to 6 mice. Mice were exposed to 850 r at 55 r per minute, delivered unfiltered from a distance of 100 cm at 230 Kv and 15 mA.

12.3 Reconstitution with bone marrow

Bone marrow donors were killed by cervical dislocation, and were pinned down on a cork board with the dorsal side uppermost and the hind limbs well stretched. The skin was swabbed with 70% alcohol and incisions were made from below the knee to the mid abdominal region on both sides. The femurs were freed from the surrounding muscle using curved scissors, and they were dislocated and removed using a combination of Michel clip forceps and scissors. The femurs were placed in a sterile petri dish containing a filter paper moistened with sterile saline.

To flush the marrow, a part of the greater trochanter was resected with scissors, and a No. 23 needle attached to a syringe was inserted through the lower extremity plateau. The bone marrow was flushed out with 2 ml of 199 medium (Wellcome) into a sterile

cavity block. The femur was flushed repeatedly until all marrow had been removed. Both femurs were flushed out with the same 2 ml of 199 medium. Each mouse yielded approximately 25×10^6 cells from two femurs, and each recipient mouse was given 0.4 ml of the cell suspension (5×10^6 cells) intravenously (I.V.) into the tail vein.

Bone marrow inocula were always given within 4 hr of x-irradiation of the recipients. After reconstitution with bone marrow, mice were placed on a filter rack (Carworth), 5 per box, and were maintained on sterile water for 20 days and given standard mouse food ad lib. supplemented with sunflower seeds.

13. THYMUS GRAFTING

(Plates 2.2a to 2.2d)

13.1 Removal of thymus lobes from neonates

Thymus graft donors were 1 to 3 day old neonates. The donor mice were killed by severing the spinal cord. They were then fixed ventral side uppermost on a strip of broad adhesive tape to facilitate removal of the thymus. The thoracic skin was dissected and a large thoracic flap resected. Using fine scissors the thymus was removed and placed in a sterile petri dish containing 199 medium. The dish was kept on ice until the grafts were used.

13.2 Technique of grafting

The technique of grafting adopted was essentially the method used by Dr Elizabeth Leuchars at the Chester Beatty Research

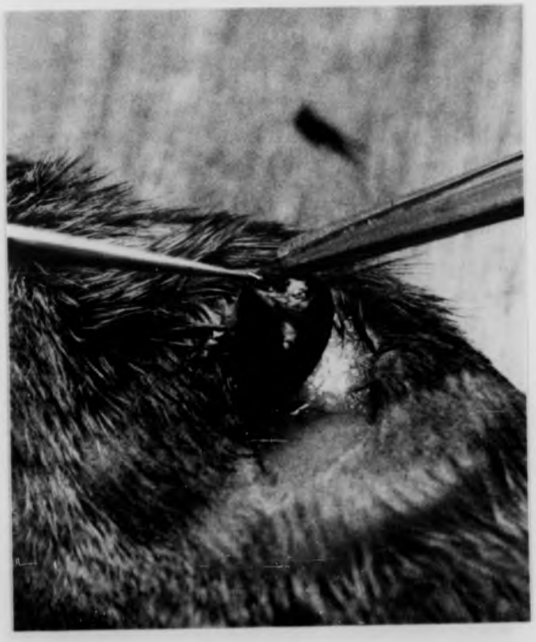
Method of Thymus Grafting

Plate 2.2a

Incision being made in body wall

Plate 2.2b

Incision being made in kidney capsule



Method of Thymus Grafting

Plate 2.2a

Incision being made in body wall

Plate 2.2b

Incision being made in kidney capsule

Plate 2.2c

Introduction of graft beneath kidney capsule

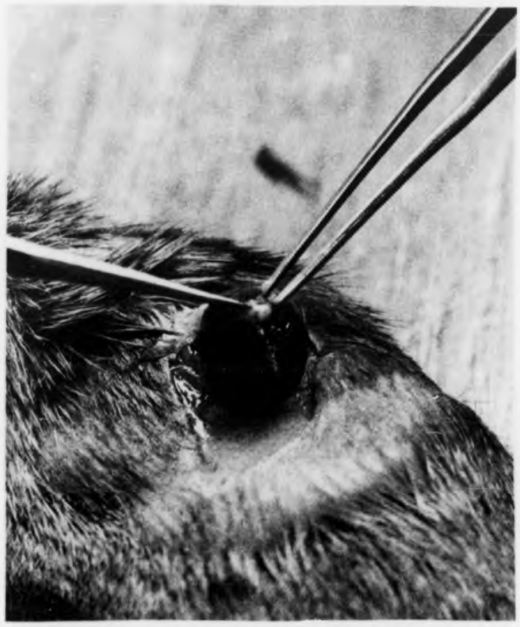


Plate 2.2d

Positioning of graft under kidney capsule

Plate 2.2c

Introduction of graft beneath kidney capsule

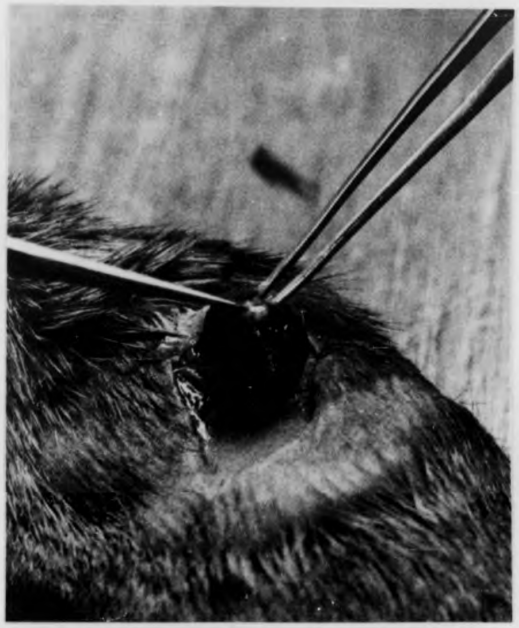


Plate 2.2d

Positioning of graft under kidney capsule

Institute, and is based on that of Law et al. (1963).

Recipient mice were initially anaesthetised with ether; anaesthesia was maintained by the use of Penthrane inhalation anaesthetic. Once anaesthetised the mice were shaved on the left flank, positioned with their left flanks uppermost, and a small incision was made in the skin over the left kidney. A second incision was made in the body wall, at the same point, using fine scissors, and the kidney was exteriorised. At this stage the thymus to be grafted was placed on the surface of the kidney. The kidney capsule was picked up with a pair of watchmaker forceps and a small incision was made in it with fine scissors. The thymus was now introduced through this opening into the space beneath the kidney capsule with a blunt forceps, and while keeping the capsule raised with watchmaker forceps, the thymus was gently slid under the capsule with the aid of the blunt forceps.

The final positioning of the graft under the capsule depended on whether the graft had to be removed later in the experiment or not. If the experiment did not require removal of the graft the thymus was positioned at the anterior end of the kidney, at some distance from the capsular incision. If the graft was to be removed it was found convenient to position the graft to one side of the incision, under the kidney capsule.

Finally, the kidney was pushed back into the peritoneal cavity, the incision in the body wall was closed with silk sutures (Dexon) and the skin incision was closed with autoclips.

14. RABBIT ANTI MOUSE THYMOCYTE SERUM

14.1 Preparation

Rabbit anti mouse thymocyte serum (ATS) was prepared according to the method of Levey and Medawar (1966). Groups of CBA/Lac mice were killed by cervical dislocation, the thymus lobes were removed, and thymocyte cell suspensions were prepared by passing the lobes through a fine wire mesh into cold 199 medium (Wellcome). The pooled cells were washed twice and resuspended in fresh 199 medium at a concentration of 1×10^8 cells/ml. 10 ml of this cell suspension was inoculated into a New Zealand white rabbit, intravenously. The procedure was repeated 7 days later and the rabbit bled 14 days after the second injection. The serum was inactivated at $56^{\circ}C$, absorbed with washed mouse RBC at $37^{\circ}C$ for 30 min (1 volume of packed RBC to 3 volumes of serum) and stored in 4 ml aliquots at $-20^{\circ}C$.

14.2 Assay for immunosuppressive potency

The immunosuppressive potency of the rabbit anti mouse thymocyte serum was assessed by its ability to prolong the survival of Parkes mice skin grafts on CBA/Lac mice. Skin grafting was performed using the technique of Bailey and Usama (1960). The grafts were examined daily and the day of rejection was taken at that on which total destruction of graft epithelium occurred. The administration of 0.2 ml doses of ATS subcutaneously, on days -1, +1, +3 and +5 in relation to the time of grafting, resulted in the pro-

longation of graft survival time to 26.4 ± 2.2 days compared to 9.7 ± 1.2 days in a normal rabbit serum (NRS) treated control group.

15. VERIFICATION OF STERILE IMMUNITY

3 to 4 weeks after recovery from a P. b. yoelii infection, blood and various tissue cell suspensions were subinoculated into clean T.O. mice, I.P.. Groups of recovered CBA mice were first bled from the retro-orbital sinus (0.5 ml of blood was collected from each mouse into heparinised tubes) and the blood was sub-inoculated immediately into T.O. mice. These mice were then killed by cervical dislocation; the spleen, liver, lungs and kidneys were removed and placed in separate tubes containing chilled 199 medium. Cell suspensions were prepared from each organ by passing the tissue through a fine wire sieve into 1 ml of 199 medium. Suspensions of bone marrow were made by flushing the contents of both femurs into 1 ml of medium. 0.5 ml quantities of these cell suspensions were then inoculated I.P. into clean T.O. mice.

16. INOCULATION OF MICE

(Intravenous inoculation)

Mice in standard mouse boxes were placed in a specially designed rectangular wooden box (30 x 30 x 70 cms) containing three 60 watt lamps, for 5 to 10 min until vasodilation occurred. Each mouse was then placed in a 'V box' (made by slitting one end of a mouse box, the upper end of the slit was 13 mm wide, the lower end

3 mm wide) and its tail held through the bottom of the slit. The tail was held with the left hand draping it over the forefinger and securing it with the thumb. A No. 25 needle was introduced into the lateral tail vein so that the needle followed directly along the line of the vein; if the needle was within the vein, no resistance was felt and inoculation could be achieved easily.

17. DATA ANALYSIS

All determinations of blood parasitaemia and nucleated cell counts (total and differential) were carried out on groups of 6 mice. Mean values and standard errors (S.E.'s) were calculated on a suitably programmed Olivetti 'Programma 101' desk computer. Where necessary Student's t-test was used to determine the significance of differences between groups.

Organ weight body weight ratios were calculated by sampling groups of 3 to 4 mice at each point in the time-course experiments reported.

The data is presented in the forms of graphs and tables. All tables are included at the end of the chapter.

EXPERIMENTS AND RESULTS

SECTION I: THE RESPONSE OF CBA MICE TO P.B. YOELII

1. GENERAL CHARACTERISTICS

1.1 The pattern of infection in normal CBA mice

Normal CBA mice infected with P. b. yoelii showed a characteristic and reproducible pattern of infection. When inoculated with 1×10^4 P. b. yoelii parasitized RBC intraperitoneally (I.P.), parasites could be detected in the peripheral blood by day 3. The parasitaemia then increased steadily, peaked on day 10 or 11 - peak parasitaemia ranging from 2 to 6% - after which there was a fairly rapid clearance of parasites from the peripheral blood. The blood was usually aparasitaemic by day 16.

1.2 Attempts to reinfect recovered mice

Mice which had recovered from a primary infection with P. b. yoelii were challenged with inocula ranging from 1×10^6 to 1×10^8 P. b. yoelii-parasitized RBC I.P.. No patent parasitaemia resulted. All parasites appeared to have been eliminated from the peripheral blood within 24 hours, as judged by blood film examination.

Two weeks after recovery from a P. b. yoelii infection groups of CBA mice were splenectomized or treated with rabbit anti mouse thymocyte serum (ATS) (see section on 'Effect of ATS' for details of treatment schedule used). No parasites were detected in the blood of these mice (as judged by the examination of blood films) after treatment, over a 20 day period of observation. 15 days after

treatment 0.5 ml of blood from ATS treated or splenectomized mice were sub-inoculated I.P. into clean 5 week old T.O. mice. No parasites were detected in the recipients over a 15 day period of observation after sub-inoculation. 15 days after splenectomy or ATS treatment, groups of treated mice and untreated recovered controls were challenged with 1×10^7 parasitized RBC. No patent infections developed in the untreated and ATS treated groups. Mice which had been splenectomized showed a transient, low grade infection which lasted 6 days.

1.3 Attempts to detect persistent parasites

To determine whether CBA mice were completely free of parasites after the clearance of the blood parasitaemia, blood and tissue cell suspensions from groups of mice 4 weeks after recovery from a primary infection, were sub-inoculated into clean T.O. mice. The tissues examined were spleen, liver, lungs, kidney and bone marrow. The recipient T.O. mice were screened for parasites, by blood film examination, for 35 days after subinoculation. No parasites were detected in recipients of blood, spleen, liver or bone marrow. 60% of those mice inoculated with kidney cell suspensions showed patent parasitaemias within 5 days of inoculation.

2. THE EFFECTS OF T CELL DEPRIVATION

To investigate the role of the T cell pool in P. b. yoelii infections the immunological responses of normal, T cell deprived (deprived) and sham-deprived (sham) mice were examined. T cell

deprived and sham deprived mice were used 50 days after x-irradiation. They were infected, together with a group of normal age-matched CBA mice, with 1×10^4 P. b. yoelii parasitized RBC I.P.. The parameters (of the host response) studied were parasitaemia, haematological changes, antibody and serum immunoglobulin levels, and histopathological changes.

In a separate experiment the effect of ATS (rabbit anti mouse thymocyte serum) on P. b. yoelii infections in normal and T cell deprived mice was investigated. In this experiment only parasitaemias were monitored.

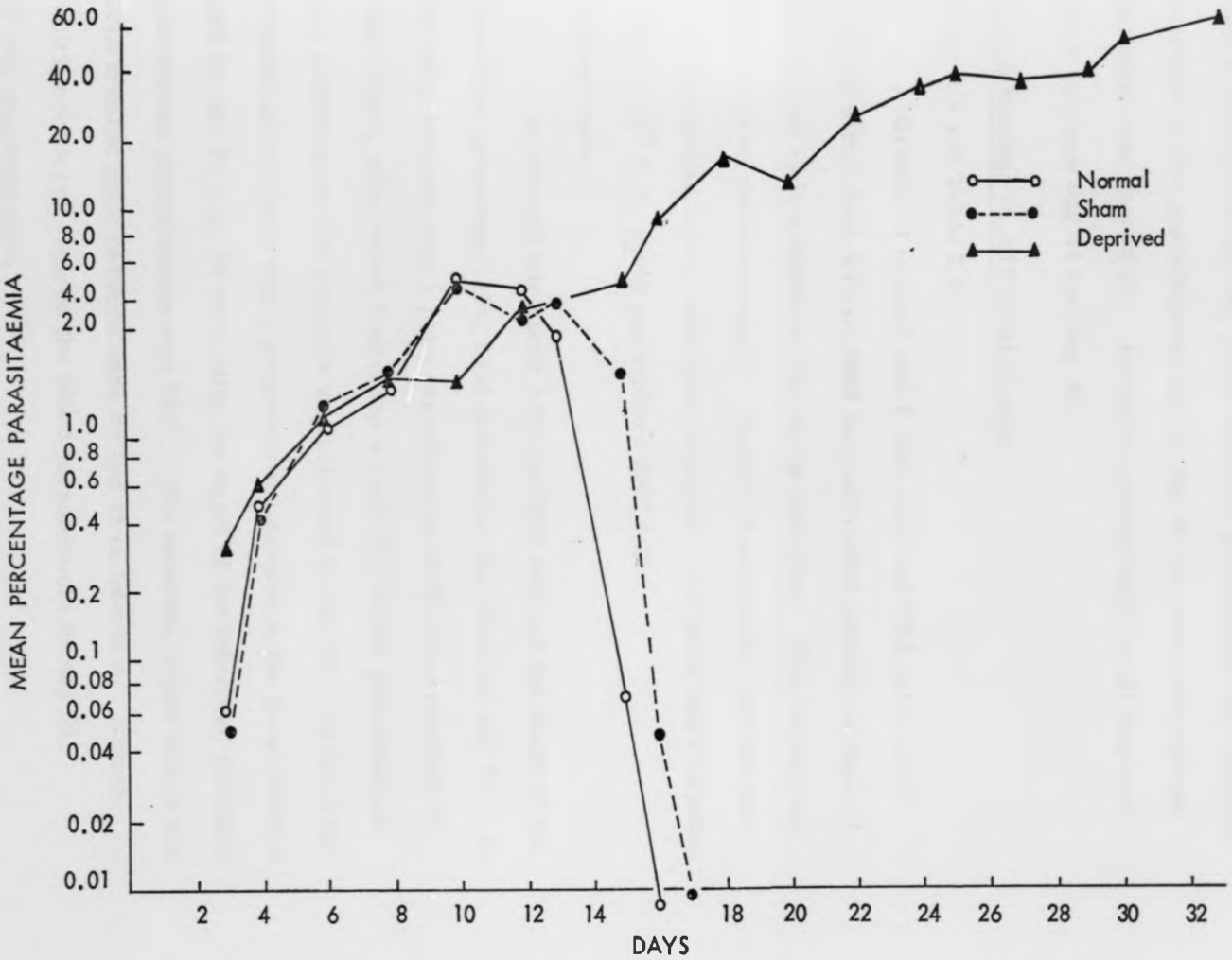
2.1 Parasitaemias

(Fig. 2.1 and Table 2.1)

Parasitaemias in normal, sham deprived and T cell deprived CBA mice

In all three groups parasites were first detected in the peripheral blood 3 days after infection. Until day 8 of the infection the parasitaemias in the deprived group did not differ significantly from those in the sham deprived or normal groups. However, on day 10 when the normal and sham groups showed peak parasitaemias of 5.15 and 5.03%, the mean parasitaemia in the deprived group (1.82%) was significantly lower ($p < 0.001$). This might suggest that at this point during the infection, the host response in the intact groups was exerting a stimulatory effect on the parasite thereby accelerating its growth. Thereafter, while in the normal and sham groups the parasitaemia regressed rapidly, the blood being

Fig. 2.1. Mean percentage parasitaemias in normal, sham deprived, and T cell deprived CBA mice infected with 1×10^4 P.b yoelii parasitized RBC.


MEAN PERCENTAGE PARASITAEMIA
60.0
40.0
20.0
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
18
20
22
24
26
28
30
32
DAYS
Normal
Sham
Deprived

aparasitaemic by day 16, in the deprived group there was a steady increase in the parasitaemia and by day 30 the mean percentage parasitaemia was 56.35. Infections proved fatal in all deprived mice between day 34 and day 40.

Parasitaemias in ATS treated mice

(Fig. 2.2 and Table 2.2)

Groups of normal and T cell deprived CBA mice were treated with 0.5 ml ATS or NRS (normal rabbit serum) on days -1, +1, +3 and +5 in relation to the day of infection. The serum was administered subcutaneously. Groups of untreated, normal and T cell deprived mice, were also included. All mice were infected with 1×10^4 P. b. yoelii parasitized RBC I.P..

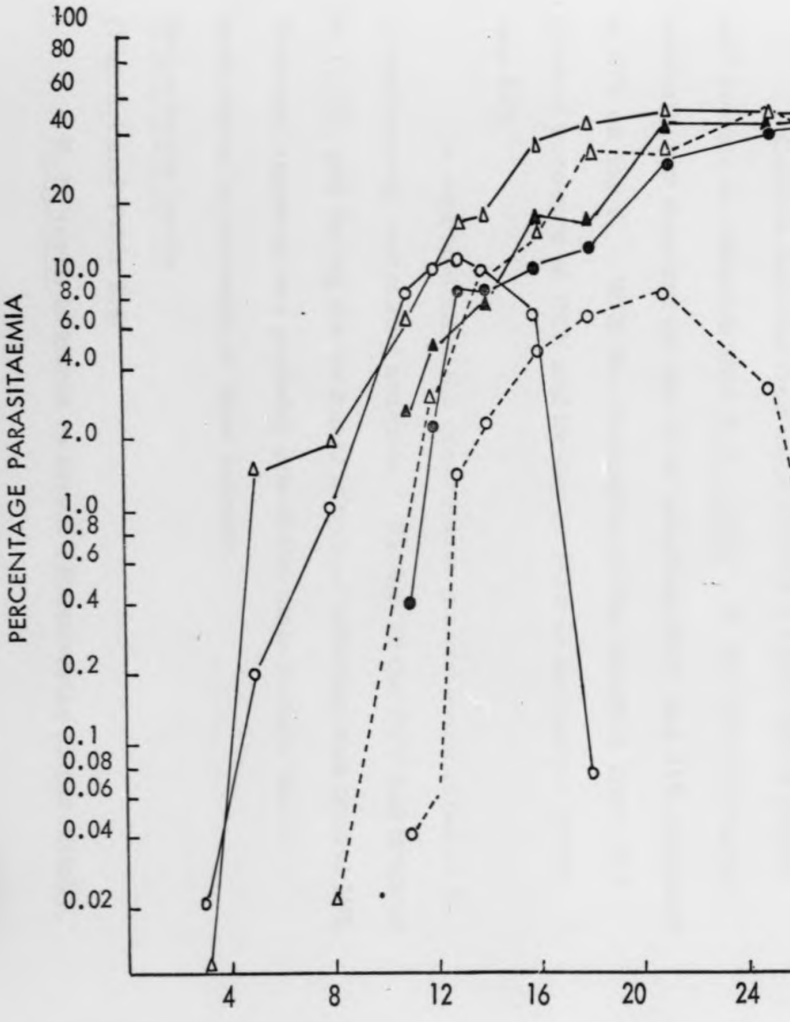
Normal mice

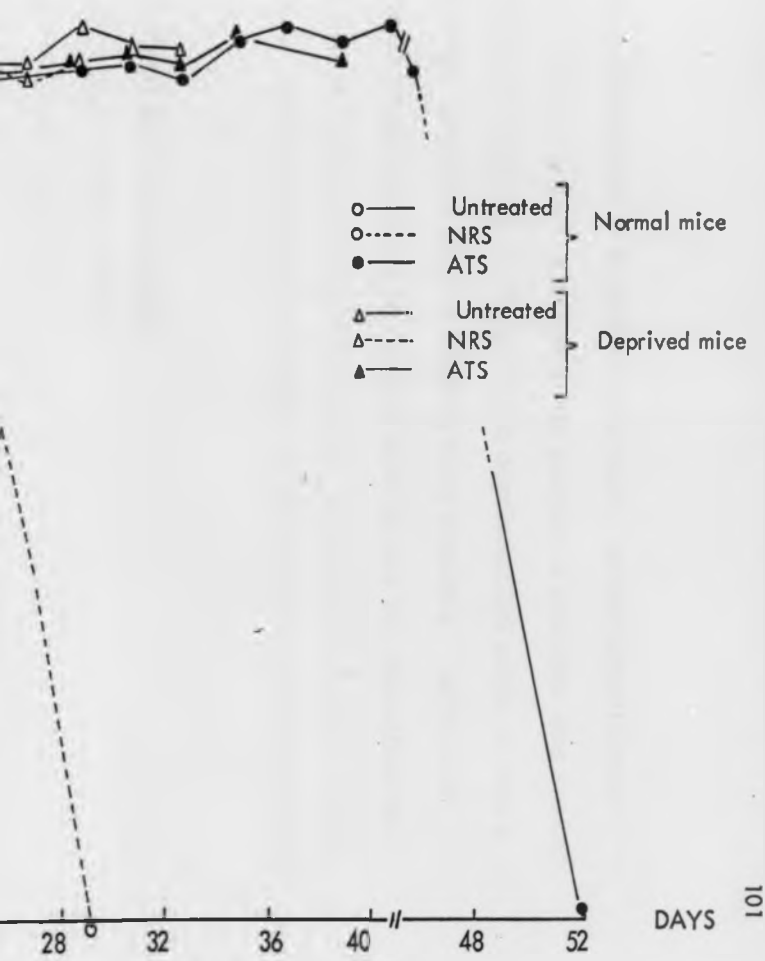
In normal mice both ATS and NRS delayed the onset of the infection, parasites being first detected in the blood on day 11. In the NRS treated mice a peak parasitaemia of 8% was recorded 10 days later, after which there was a rapid fall in the parasitaemia and clearance of the parasite was achieved by day 29. In the ATS treated group there was a progressive increase in the parasitaemia and by day 35 (i.e. 25 days after the onset of the infection) the mean percentage parasitaemia was 55.7. The infection killed 70% of the ATS treated mice between days 36 and 46 of infection. Those which recovered cleared the blood parasitaemia by day 52.

T cell deprived mice

ATS and NRS treated deprived mice also showed extended

Fig. 2.2 Mean percentage parasitaemias in rabbit anti mouse thymocyte serum (ATS) treated normal and T cell deprived mice, and in normal rabbit serum (NRS) treated normal and T cell deprived mice. Inoculum 1×10^4 P.b. yoelii parasitized RBC.


PERCENTAGE PARASITAEMIA
100
80
60
40
20
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
4
8
12
16
20
24


Untreated
NRS
ATS
Normal mice
Untreated
NRS
ATS
Deprived mice
28
32
36
40
48
52
DAYS

prepatent periods. In NRS treated mice, patent parasitaemias were seen on day 8 - thereafter the pattern of infection was similar to that seen previously in untreated deprived CBA mice, in that a fulminant infection which killed all mice resulted. In the ATS treated group parasites were first seen on day 11, thereafter the pattern of infection was similar to that observed in the NRS treated group. The infection proved fatal in these mice between day 34 and 39.

2.2 Haematological changes

Packed cell volume (PCV)

(Fig. 2.3 and Table 2.3)

Control and deprived mice showed a rapid fall in packed cell volume on infection with P. b. yoelii. In the control groups anaemia was maximal on day 12 of infection (PCV was 31% compared to 47% on day 0). With the elimination of the infection there was gradual recovery of PCV and by day 21 PCV in the control group was 44%.

In deprived mice the progressive parasitaemia resulted in a continuously increasing anaemia. By day 22 the PCV had dropped to 17.5%, and during the terminal stages of infection was around 10%. Profound anaemia was probably one of the main factors which contributed to the death of these animals.

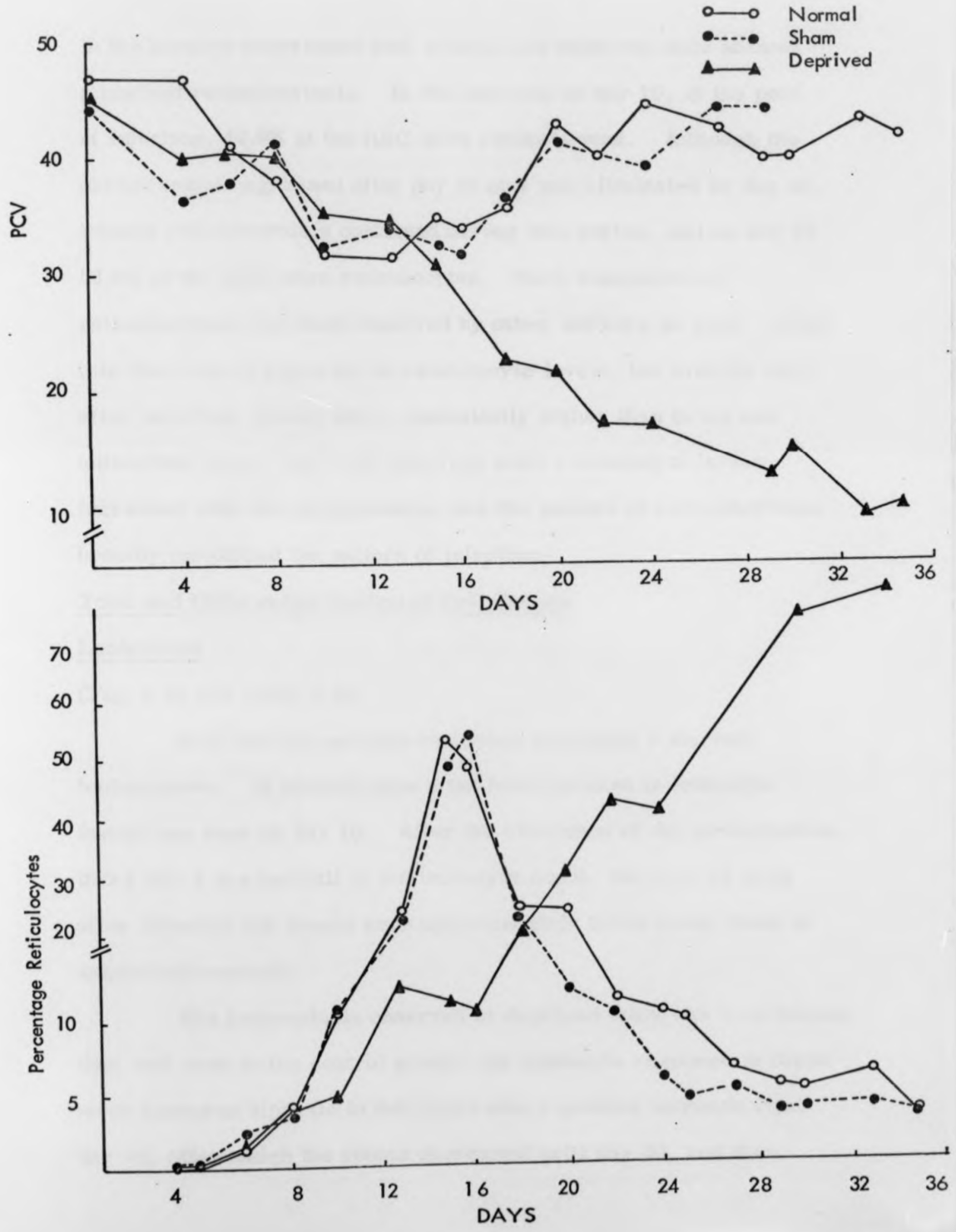
Reticulocyte levels

(Fig. 2.4 and Table 2.4)

P. b. yoelii is known to invade reticulocytes preferentially.

Fig. 2.3 Packed cell volume (PCV) in normal, sham and T cell deprived CBA mice infected with P.b. yoelii

Fig. 2.4 Mean percentage reticulocyte levels in normal, sham and T cell deprived mice infected with P.b. yoelii.


Normal
Sham
Deprived
PCV
50
40
30
20
10
4
8
12
16
20
24
28
32
36
DAYS
Percentage Reticulocytes
70
60
50
40
30
20
10
5
4
8
12
16
20
24
28
32
36
DAYS

In the present experiment both control and deprived mice showed a marked reticulocytosis. In the controls on day 10, at the peak of infection, 10.9% of the RBC were reticulocytes. Although the parasitaemia regressed after day 10 and was eliminated by day 16, intense reticulocytosis continued during this period, and on day 15 53.6% of the RBC were reticulocytes. Such compensatory reticulocytosis has been observed by other workers as well. After this there was a rapid fall in reticulocyte levels, but even 30 days after infection, levels were substantially higher than in normal uninfected mice. In T cell deprived mice reticulocyte levels increased with the parasitaemia, and the pattern of reticulocytosis broadly paralleled the pattern of infection.

Total and Differential Nucleated Cell Counts

Leukocytes

(Fig. 2.5a and Table 2.5a)

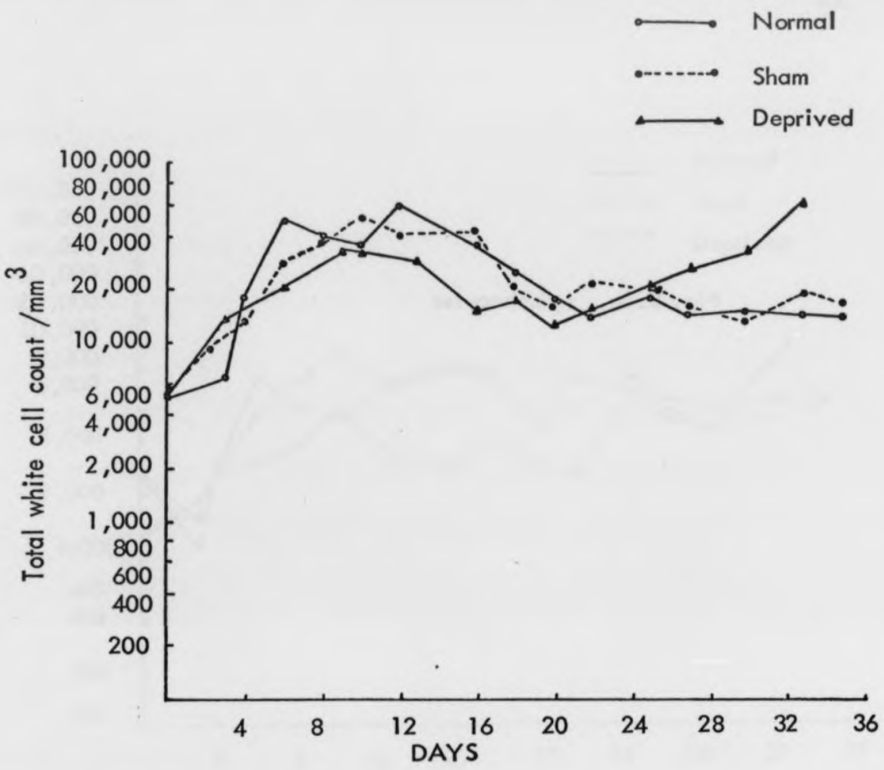
Both normal and deprived mice developed a marked leukocytosis. In normal mice a ten fold increase in leukocyte levels was seen by day 10. After the clearance of the parasitaemia, there was a gradual fall in the leukocyte count, but even 35 days after infection the counts were approximately three times those in uninfected controls.

The leukocytosis observed in deprived mice was less intense than that seen in the control group; the leukocyte response in these mice appeared biphasic in that there was a gradual increase until day 10, after which the counts decreased until day 20, and then

Fig. 2.5 Absolute numbers of nucleated cells per mm^3 in the peripheral blood of normal, sham and T cell deprived mice infected with P.b. yoelii

Fig. 2.5a Total nucleated cell count

Fig. 2.5b Neutrophils


Normal
Sham
Deprived
Total white cell count /mm3
100,000
80,000
60,000
40,000
20,000
10,000
6,000
4,000
2,000
1,000
800
600
400
200
4
8
12
16
20
24
28
32
36
DAYS

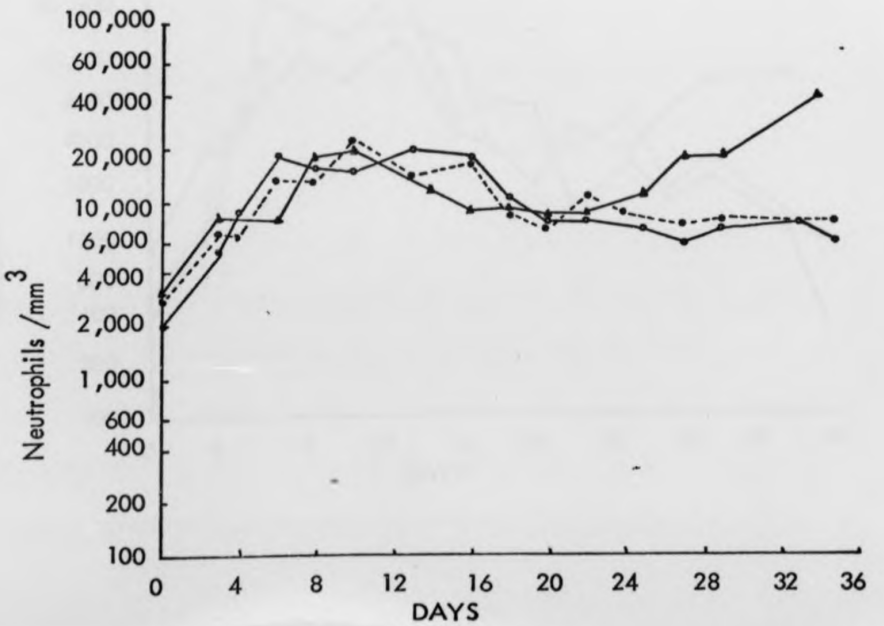
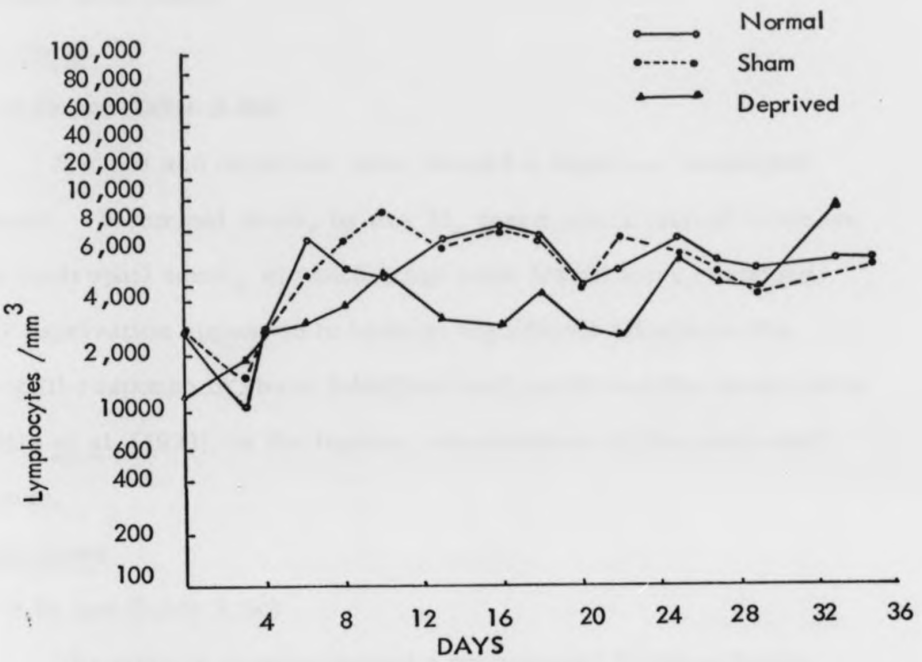
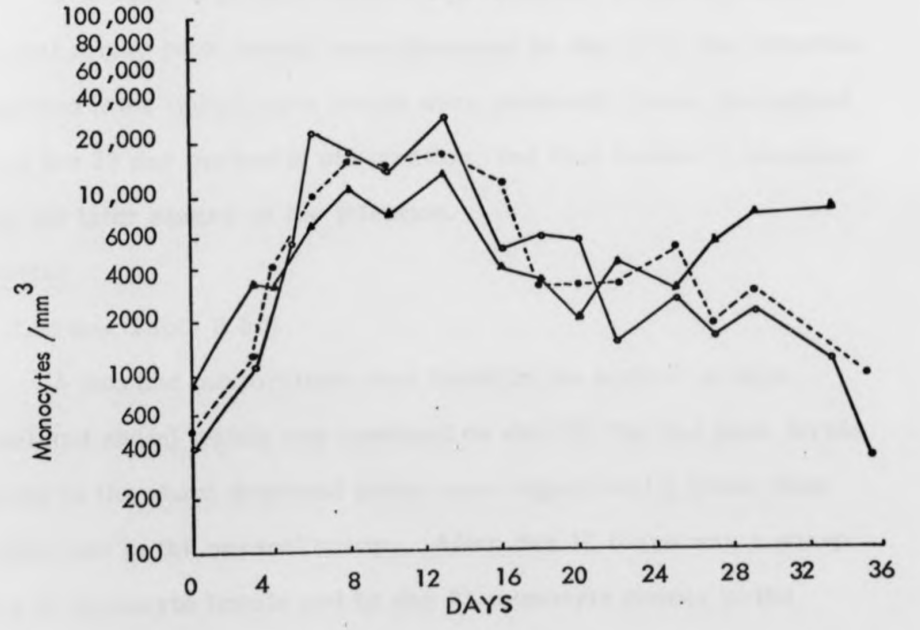

Neutrophils /mm3
100,000
60,000
40,000
20,000
10,000
6,000
4,000
2,000
1,000
600
400
200
100
0
4
8
12
16
20
24
28
32
36
DAYS

Fig. 2.5c Lymphocytes

Fig. 2.5d Monocytes


Normal
Sham
Deprived
100,000
80,000
60,000
40,000
20,000
10,000
8,000
6,000
4,000
2,000
10000
600
400
200
100
Lymphocytes /mm3
4
8
12
16
20
24
28
32
36
DAYS


100,000
80,000
60,000
40,000
20,000
10,000
6000
4000
2000
1000
600
400
200
100
Monocytes /mm3
0
4
8
12
16
20
24
28
32
36
DAYS

increased once more.

Neutrophils

(Fig. 2.5b and Table 2.5b)

Normal and deprived mice showed a vigorous neutrophil response. In normal mice, by day 23, there was a tenfold increase in the neutrophil count, at which stage peak levels were recorded. T cell deprivation appeared to have no significant effects on the neutrophil response in these infections and confirmed the observation of Walls et al. (1971) on the thymus independence of the neutrophil response.

Lymphocytes

(Fig. 2.5c and Table 2.5c)

The control groups showed a pronounced lymphocytosis. In normal mice, peak levels were observed on day 16 of the infection. In deprived mice lymphocyte levels were generally lower throughout most of the 33 day period of observation, but they tended to increase during the later stages of the infection.

Monocytes

(Fig. 2.5d and Table 2.5d)

A marked monocytosis was found in the control groups (normal and sham) which was maximal on day 13, but the peak levels recorded in the sham deprived group were significantly lower than that observed in the normal group. After day 13 there was a sharp decline in monocyte levels and by day 35 monocyte counts in the control groups were within the normal range. The monocyte

response in deprived mice appeared to be seriously impaired, monocyte levels being substantially lower than the controls throughout most of the infection.

2.3 Immunopathology

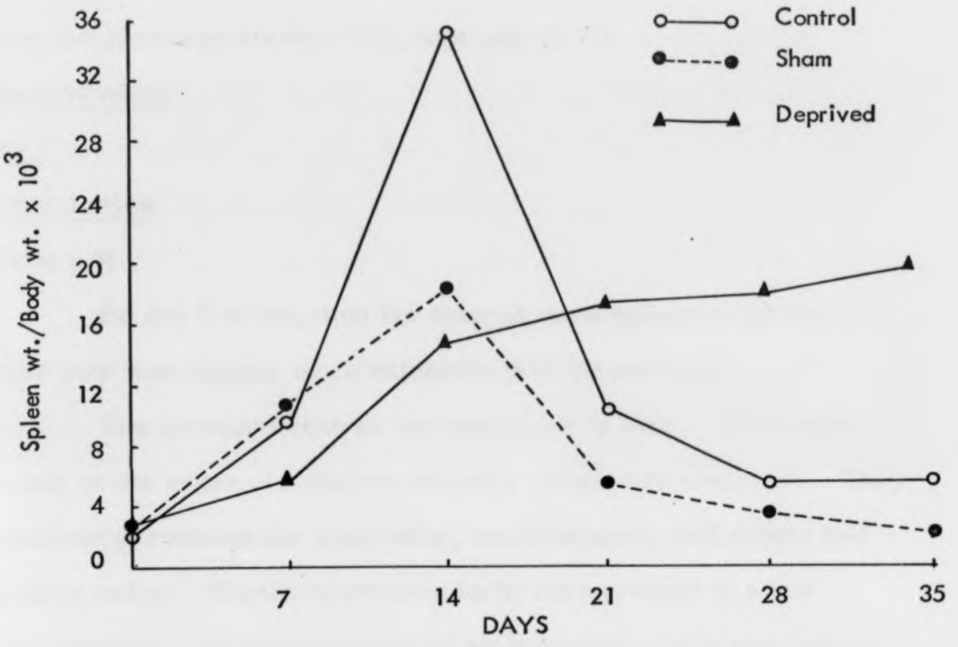
To study the histopathological changes during P. b. yoelii infections groups of normal, sham and deprived mice were sacrificed on day 0, 7, 14, 21, 28 and 35 of infection, and the spleen, lymph nodes (axillary and mesenteric), Peyer's patches, thymus, liver, lungs, kidneys and bone marrow were removed and fixed for histological examination. Before the animals were sacrificed body weights were recorded. Spleen and axillary lymph node weights were also obtained before these tissues were fixed. No significant differences in histopathology were observed between the normal and sham deprived mice. Hence the description of the histological changes has been limited to the normal and T cell deprived groups.

A. Spleen

Spleen weight/ body weight ratio

(Fig. 2.6 and Table 2.6)

In intact mice the spleen weight:body weight ratio showed a rapid increase and by day 14 it had increased from 2.80 to 35.40. With the eradication of the infection there was an equally fast drop, and by day 28 it was 5.60. Sham deprived mice showed a similar pattern of response but the peak value recorded on day 14 (18.60) was significantly less than in the normal group. Deprived mice showed a gradual increase in spleen weight which paralleled the


Control
Sham
Deprived
Spleen wt./Body wt. x 10^3
36
32
28
24
20
16
12
8
4
0
7
14
21
28
35
DAYS

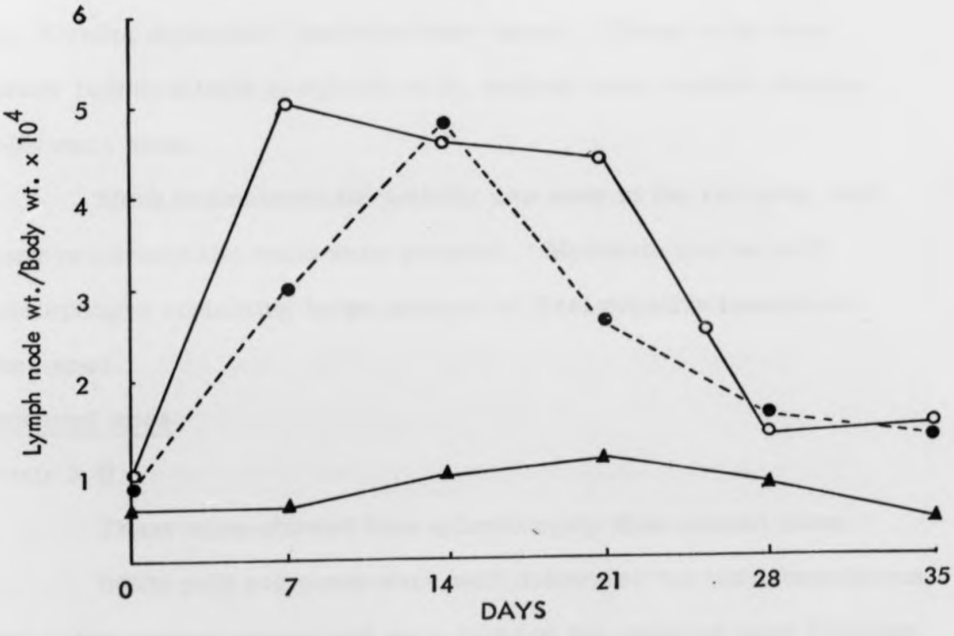
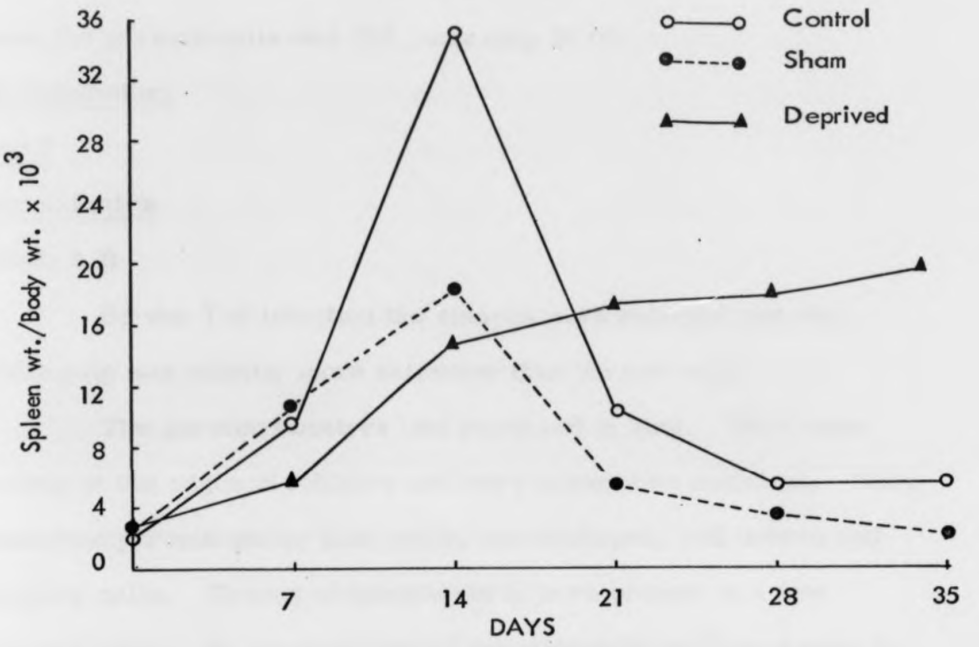
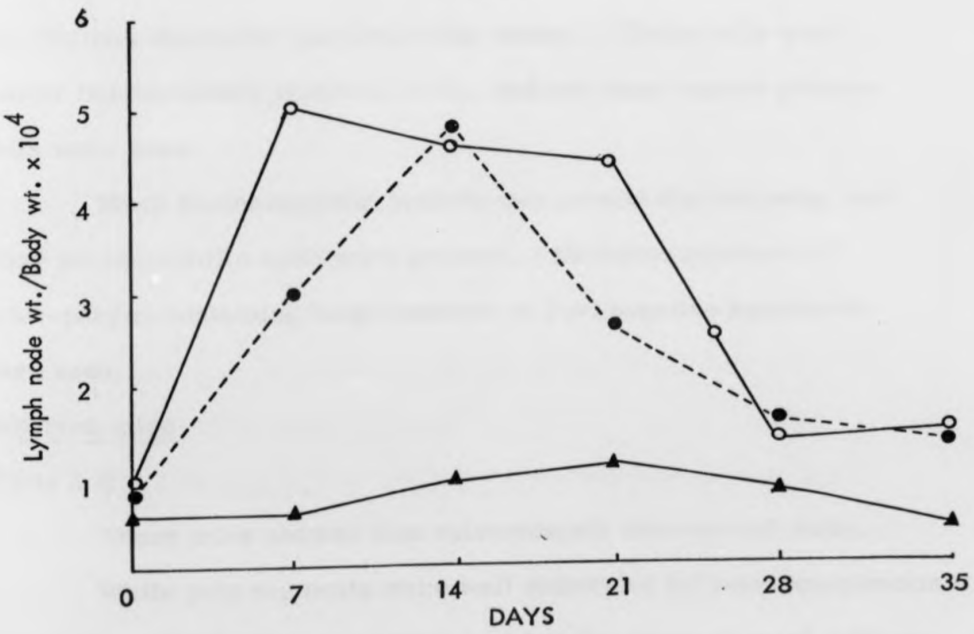

Lymph node wt./Body wt. x 10^4
6
5
4
3
2
1
0
7
14
21
28
35
DAYS

Fig. 2.6 Spleen weight/body weight ratio in normal, sham and T cell deprived mice infected with P.b. yoelii

Fig. 2.7 Axillary lymph node weight/body weight ratio in normal, sham and T cell deprived mice infected with P.b. yoelii .


Control
Sham
Deprived
Spleen wt./Body wt. x 10^3
36
32
28
24
20
16
12
8
4
0
7
14
21
28
35
DAYS


Lymph node wt./Body wt. x 10^4
6
5
4
3
2
1
0
7
14
21
28
35
DAYS

pattern of infection but, the maximum values recorded on day 35, when the parasitaemia was 79%, was only 20.00.

Histopathology

Day 7

Normal mice

(Plate 2.3)

By day 7 of infection the spleens were enlarged and the white pulp was slightly more extensive than the red pulp.

The germinal centres had increased in size. They were mainly at the edges of follicles and were sometimes confluent. They contained pyroninophilic blast cells, macrophages, cell debris and dividing cells. Traces of haemosiderin were present in a few macrophages. An accumulation of pyroninophilic cells was seen in the 'thymus dependent' periarteriolar zones. These cells were mainly indeterminate lymphoid cells, and not many mature plasma cells were seen.

Much haematopoietic activity was seen in the red pulp, and many pyroninophilic cells were present. Moderate numbers of siderophages containing large amounts of Perl negative haemozoin were seen.

Deprived mice

(Plate 2.4)

These mice showed less splenomegaly than normal mice.

White pulp segments were well delineated but less conspicuous than in the control groups and were found at the edges of most follicles.

They contained the same constituents as in the normal mice. The macrophages contained little haemosiderin. There was some accumulation of pyroninophilic cells around the central arteriole but it was less extensive than in the normal mice; the outer parts of the periarteriolar region were somewhat hypocellular. The reticulin framework was normal.

There was considerable haematopoietic activity in the red pulp. Many pyroninophilic cells and siderophages loaded with haemozoin were present.

Day 14

Normal mice

(Plate 2.5)

Splenomegaly was pronounced and maximal at this stage. The red pulp was more extensive than the white pulp.

In the white pulp active looking germinal centres were present at the edges of the follicles. The constituents were the same as at day 7. No iron pigment was seen in the macrophages. The periarteriolar regions were now stacked with pyroninophilic cells. A few appeared to be mature plasma cells. The pyroninophilic cell response was maximal at this stage. The reticulin framework was somewhat expanded.

The red pulp showed massive hyperplasia and there was more haematopoietic activity than at day 7. There was an increase in the number of pyroninophilic precursor cells, and of siderophages which were filled with large granules of haemozoin. A few mature

plasma cells were present.

The morphological changes in the red pulp appeared to be greatest at this stage.

Deprived mice

These spleens were much smaller than in normal mice at the same stage of infection. The red pulp was slightly more extensive than the white pulp.

The white pulp was not as active as in the normal mice, and the germinal centres were much smaller. The zone of pyroninophilic cells around the central arteriole was more prominent than at day 7, but was still less extensive than in the normal mice. The reticulin framework was intact.

The red pulp showed increased haematopoiesis and contained numerous siderophages loaded with haemozoin.

Day 21

Normal mice

(Plate 2.6)

By day 21 the splenomegaly was much reduced.

The white pulp was now slightly more extensive than the red pulp. The germinal centres were large and very active looking. They contained much debris and a little Perl positive iron pigment. Some centres were indistinct and merged with the pyroninophilic cells cuffing the central arteriole. The zone of pyronin positive cells showed the same constituents as at day 14. The reticulin framework was normal at this stage.

In the red pulp activity was waning. Many siderophages were present.

Deprived mice

(Plate 2.7)

On day 21 the 'deprived' spleens were larger than the 'normal' spleens.

The white pulp segments were inconspicuous, and small, poorly delineated germinal centres were present at the edges of the follicles. The zone of pyroninophilic lymphoid cells adjacent to the central arteriole was quite extensive by this stage. It consisted of mixed mature plasma cells and other forms. Most pyroninophilic cells in the white pulp were concentrated in this region at day 21. No periarteriolar depletion was evident.

Massive haematopoietic activity was seen in the red pulp, and was comparable to that seen in normal mice on day 14. Many siderophages containing large Perl negative granules were present.

Day 28

Normal mice

(Plate 2.8)

The spleens contained large white pulp segments with numerous well defined germinal centres. They contained much debris and some pyroninophilic cells. The macrophages contained faint traces of haemosiderin. The central arterioles were no longer prominent and the periarteriolar pyroninophilic cells were less conspicuous and completely absent from some follicles.

The red pulp showed declining haematopoietic activity. It was congested with haemozoin containing siderophages, and looked like a spleen soon after carbon injection.

Deprived mice

(Plates 2.9 and 2.10)

At day 28 the red pulp was much more extensive than the white pulp, the white pulp being almost completely submerged in a hyperplastic red pulp. A few residual germinal centres still persisted. Extensive cuffs of pyroninophilic cells which included mature plasma cells were found around the central arterioles. Often they were the only traces of a follicle. No periarteriolar depletion was seen. The reticulin framework was now shrunken and condensed round the central arteriole.

The red pulp showed intense hyperplasia and was congested with many pyroninophilic precursor cells and haemozoin containing siderophages.

Day 35

Normal mice

35 days after infection the germinal centres were still markedly enlarged and active looking. They contained abundant debris and macrophages. Few periarteriolar pyroninophilic cells were seen at this stage. Often they were absent altogether.

Haematopoietic activity in the red pulp was not increased above normal levels, but many haemozoin containing siderophages were still present.

The red pulp showed declining haematopoietic activity. It was congested with haemozoin containing siderophages, and looked like a spleen soon after carbon injection.

Deprived mice

(Plates 2.9 and 2.10)

At day 28 the red pulp was much more extensive than the white pulp, the white pulp being almost completely submerged in a hyperplastic red pulp. A few residual germinal centres still persisted. Extensive cuffs of pyroninophilic cells which included mature plasma cells were found around the central arterioles. Often they were the only traces of a follicle. No periarteriolar depletion was seen. The reticulin framework was now shrunken and condensed round the central arteriole.

The red pulp showed intense hyperplasia and was congested with many pyroninophilic precursor cells and haemozoin containing siderophages.

Day 35

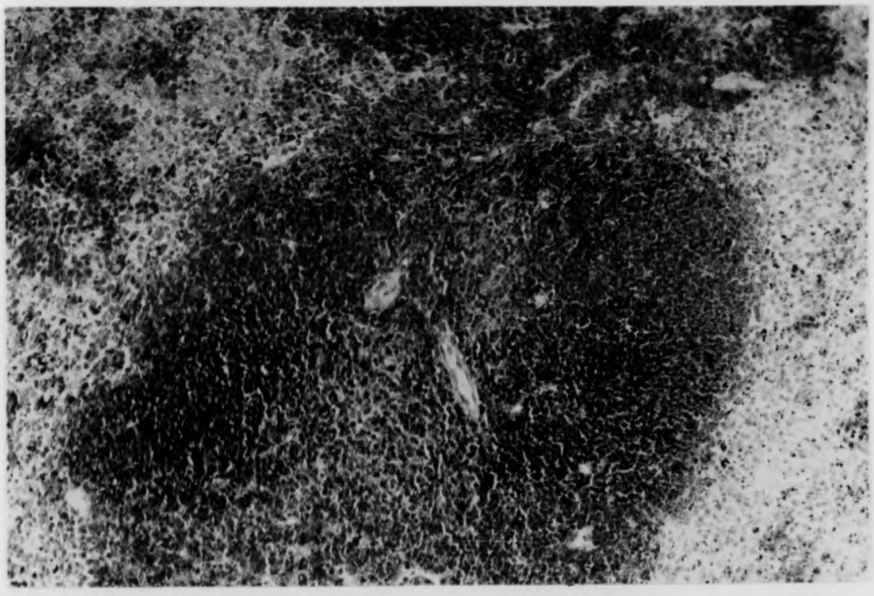
Normal mice

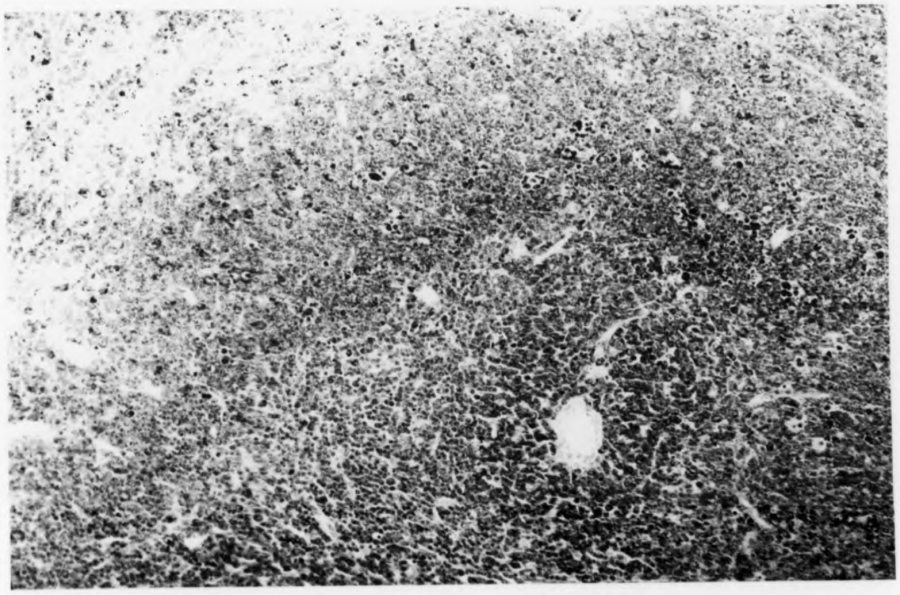
35 days after infection the germinal centres were still markedly enlarged and active looking. They contained abundant debris and macrophages. Few periarteriolar pyroninophilic cells were seen at this stage. Often they were absent altogether.

Haematopoietic activity in the red pulp was not increased above normal levels, but many haemozoin containing siderophages were still present.

Plate 2.3 Normal CBA mouse; day 7 P. b. yoelii infection. Central arteriole of spleen surrounded by an accumulation of pyroninophilic cells. Methyl green Pyronin (M.G.P.) x 196

Plate 2.4 Deprived mouse; day 7 P. b. yoelii infection. Spleen showing a white pulp segment, with germinal centres at the edges of the follicles. A few pyroninophilic cells are present around the central arteriole.





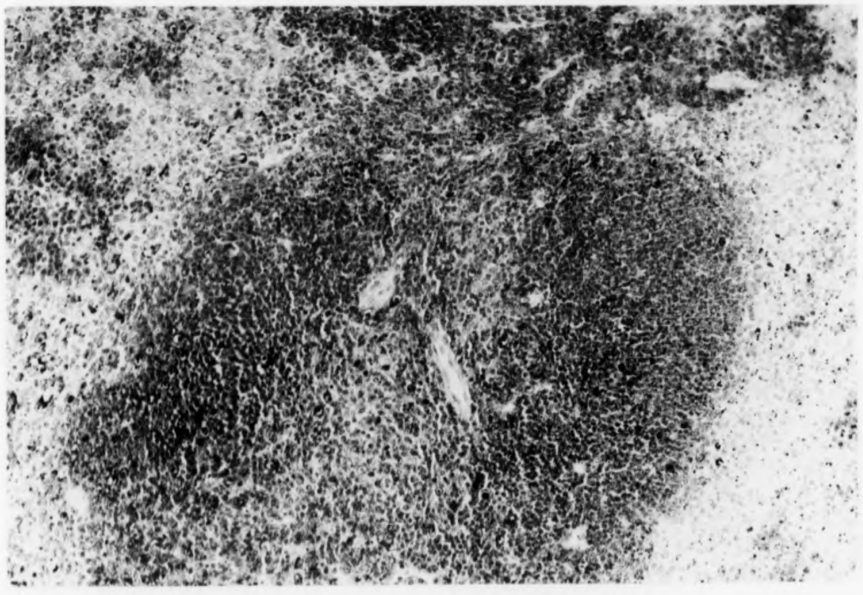
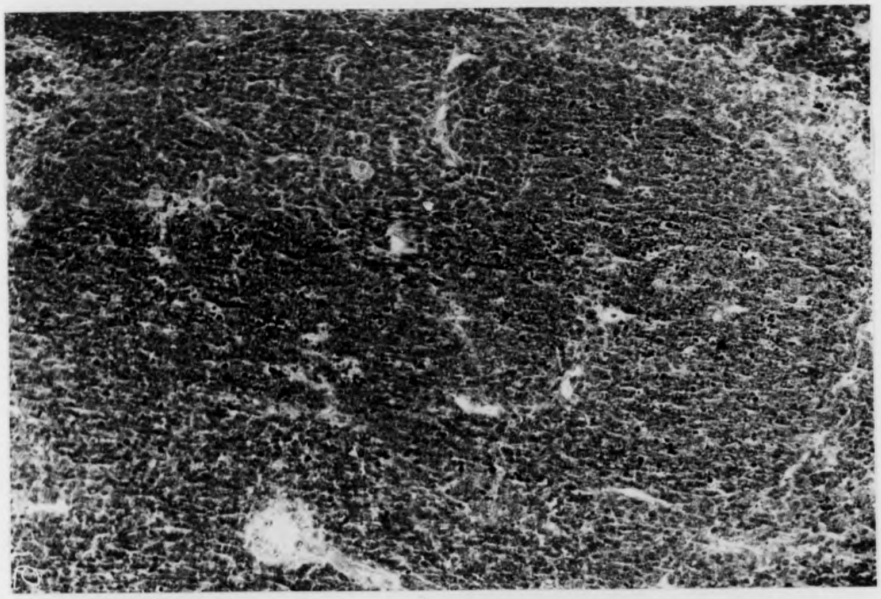


Plate 2.5 Normal CBA mouse; day 14 P. b. yoelii infection. White pulp of spleen showing active germinal centres along the edges of the follicles. An extensive zone of pyroninophilic cells is present around the central arteriole. M.G.P. x196



infection.
inal
An
present
196

Plate 2.6 Normal mouse; day 21 P. b. yoelii infection. White pulp of spleen showing a large and active looking germinal centre. M.G.P. x 235

Plate 2.7 Deprived mouse; day 21 P. b. yoelii infection. Spleen showing poorly demarcated white pulp segments; small germinal centres are present at the edges of the follicle; an accumulation of pyroninophilic cells is present around the central arteriole.

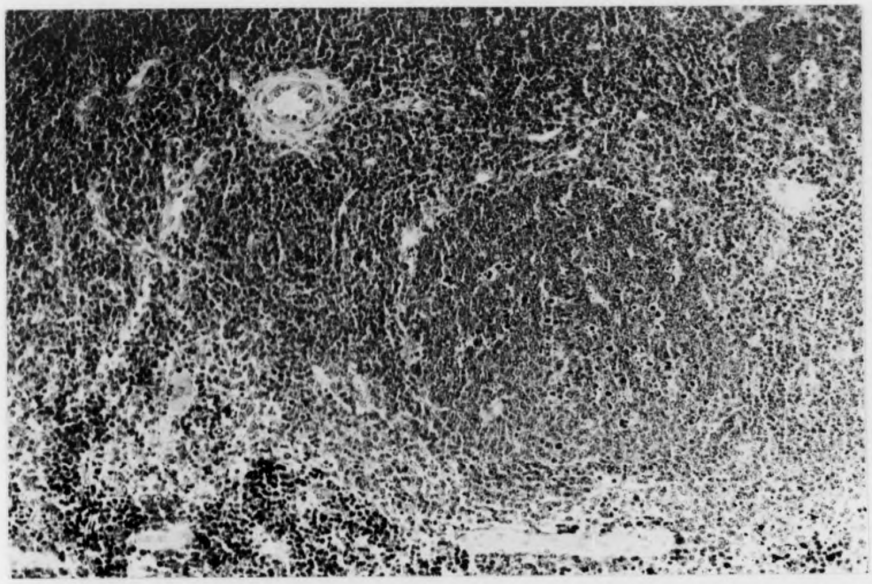
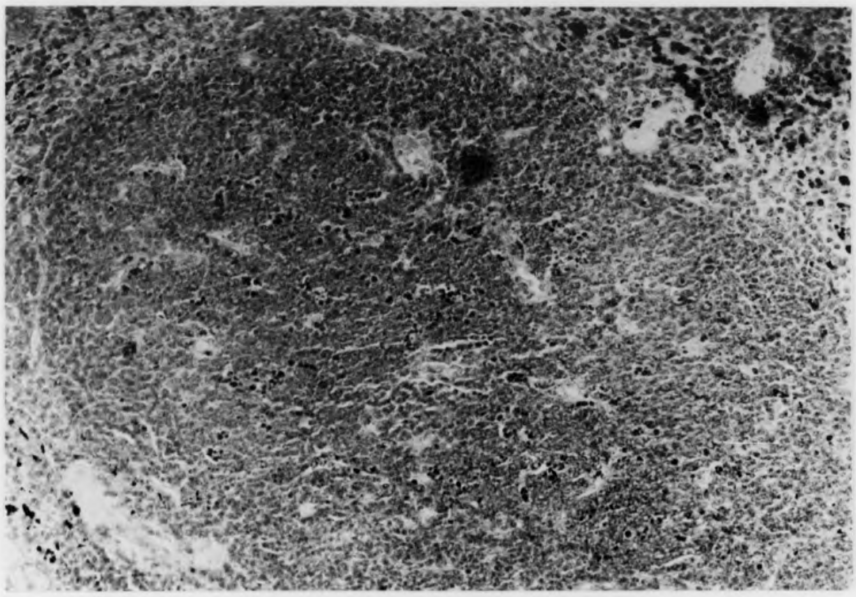


Plate 2.8 Normal CBA mouse; day 28 P. b. yoelii infection. White pulp segment of spleen showing large germinal centres. The central arteriole is not prominent and few pyroninophilic cells surround it. M.G.P. x235

Plate 2.9 Deprived mouse; day 28 P. b. yoelii infection. Spleen showing small white pulp segments and hyperplastic red pulp. An extensive zone of pyroninophilic cells is found cuffing the central arteriole. M.G.P. x235



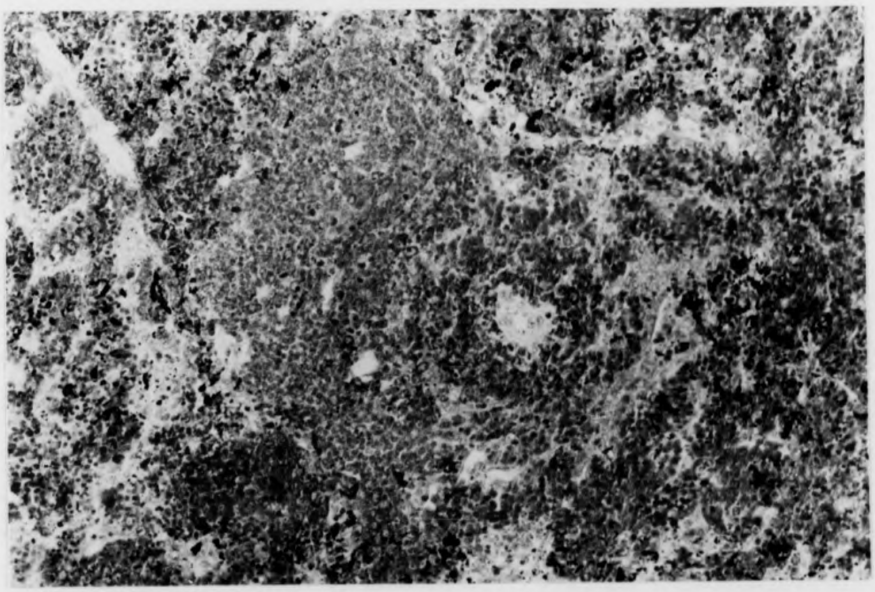
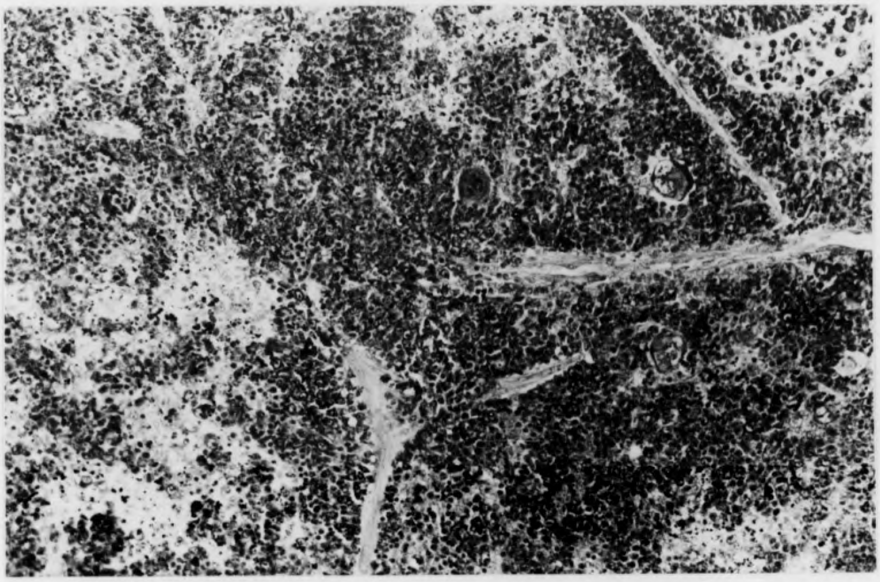


Plate 2.10 Deprived mouse; day 28 P. b. yoelii infection. Hyperplastic red pulp of spleen containing many pyroninophilic cells, and macrophages loaded with haemozoin. M.G.P. x 196

Plate 2.11 Deprived mouse; day 35 P. b. yoelii infection. Spleen showing extensive red pulp and poorly demarcated white pulp. The pyroninophilic cell infiltrate around the central arteriole is maximal. No organised germinal centres are present. M.G.P. x 196



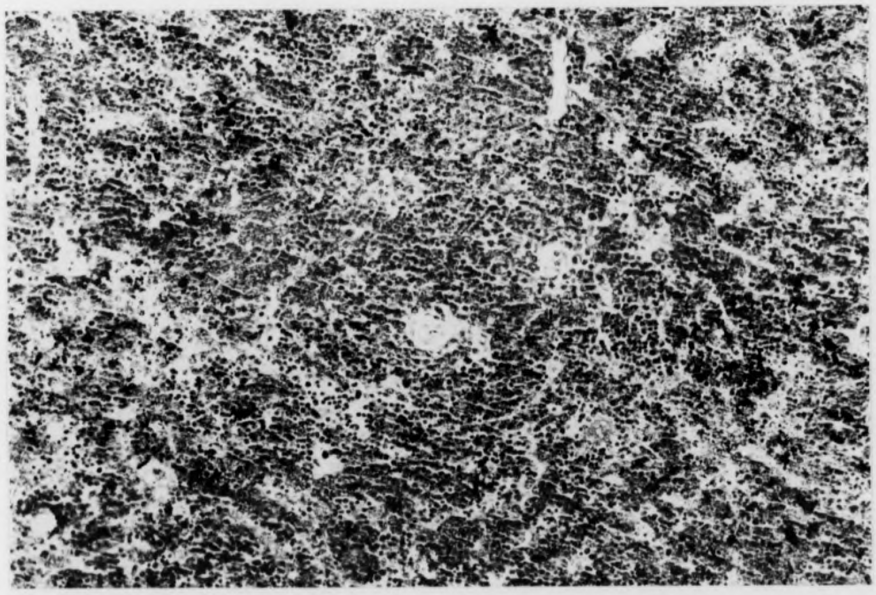
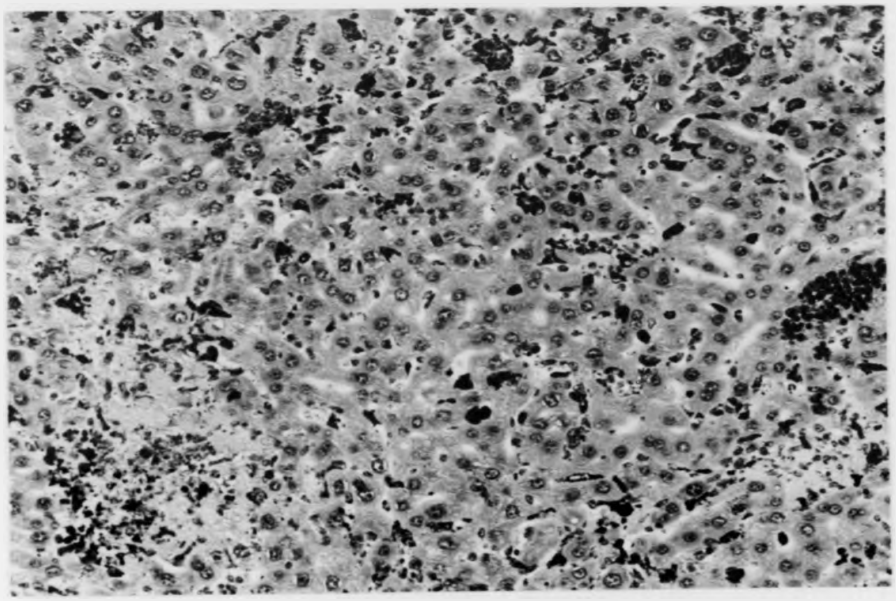
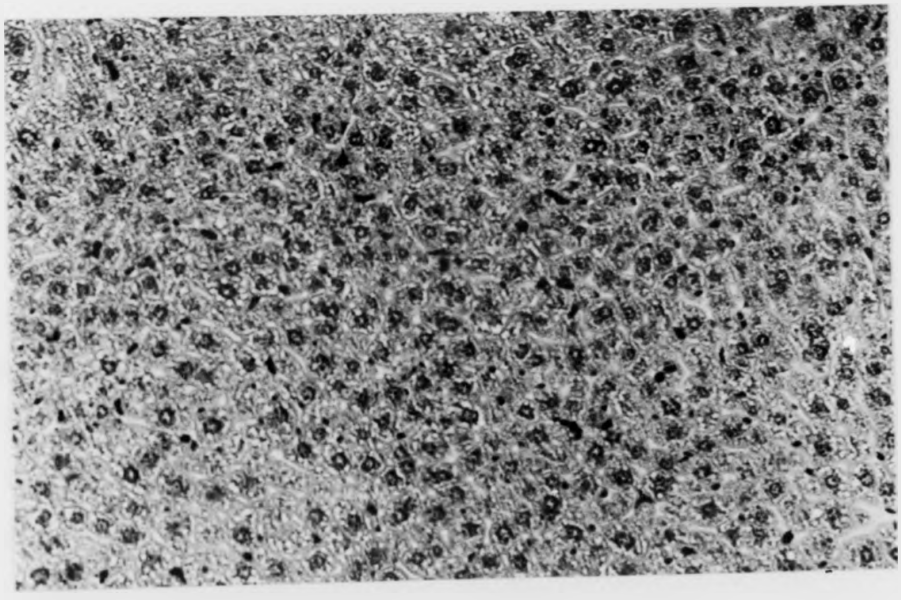


Plate 2.12 Liver of deprived mouse; day 28 of *P. b. yoelii* infection. Much pigment is present within the Kupffer cells. Foci of hepatic necrosis are present. M.G.P. x235

Plate 2.13 Liver of normal mouse; day 28 of *P. b. yoelii* infection. Hepatocytes showing cytoplasmic vacuolation.





Deprived mice

(Plate 2.11)

During the terminal stages of the infection, in the deprived mice the spleens were dominated by an extensive red pulp.

The white pulp consisted of vestigial follicles with virtually no organised germinal centres. The infiltration of the periarteriolar area with pyroninophilic cells which had increased as the infection progressed was now maximal. The pyronin positive zone, the outer borders of which were ill-defined contained some mature plasma cells and there was no evidence of any periarteriolar depletion. The reticulin framework had collapsed by this stage.

There was continuing haematopoiesis in the red pulp with much pyroninophilia. Large numbers of pigment laden macrophages were present and tended to outline the white pulp segments.

B. Lymph Nodes (Axillary and Mesenteric)

Axillary lymph node weight/ body weight ratio

(Fig. 2.7 and Table 2.7)

Both normal and sham deprived mice showed a substantial increase in the axillary lymph node weight : body weight ratio. In normal mice a five fold increase was observed by day 7, and it remained significantly increased until day 21. In the sham controls the response was slower and peak values (4.99) were observed on day 14 after which there was a gradual decrease. Deprived mice showed little change in lymph node weight, the ratio increasing from 0.6 on day 0 to a maximum of 1.10 on day 21.

Histopathology

Although a substantial increase in the axillary lymph node weight was recorded in control groups, histologically no reactive changes were evident in the axillary or mesenteric lymph nodes of normal or deprived mice. The deprived mice showed the usual paracortical hypoplasia.

C. Peyer's Patches

No significant reactive changes were seen in the Peyer's patches of normal or deprived mice between days 7 and 35 of infection. Some peripheral cellular depletion was observed in the patches from deprived mice.

D. Thymus

No changes were observed in the thymus of normal or sham controls over the 35 day period of study.

E. Liver

(Plates 2.12 and 2.13)

Pigment was seen in the Kupffer cells of the liver from day 7 onwards in both normal and deprived mice. The amount of pigment was about equal at 7 and 14 days, thereafter it was greater in the deprived mice. The pigment was almost entirely negative with Perl's stain and hence was haemozoin rather than haemosiderin.

The hepatocytes showed cytoplasmic vacuolation at and after day 14 in both normal and deprived mice. Some pigment loading of hepatocytes was seen at day 14. Subsequently it decreased in normal mice but increased in deprived mice. Small foci of hepatic necrosis

were seen in deprived mice at day 28.

Extramyeloid haematopoiesis was observed in normal and deprived mice at day 14. It increased in deprived mice after day 14.

F. Kidneys

By day 7 of infection most glomerular tufts were swollen, filling Bowman's capsule. The tufts were not hypercellular. There was no increase in PAS (Periodic Acid Schiff) positive material which showed a mesanglial distribution. PAS positive thickenings of capillary loops were also seen, and these appeared dilated in some instances. Some debris was seen in the glomerular capillaries. Pigment was present in the glomerular capsule, the epithelium of the proximal convoluted tubules (especially along the luminal border), free in the tubular lumen and also in the interstitial connective tissue. Little or no pigment was seen in the glomerular tufts. The proximal convoluted tubules were the first affected in both normal and deprived mice at day 14. Thereafter the pigment increased in deprived mice and decreased in normal mice. Most of the pigment gave a positive Perl's reaction and was therefore largely haemosiderin.

G. Bone Marrow

Haematopoietic elements appeared to be increased by day 7 filling the marrow spaces. Thereafter persistent haematopoiesis was seen in the deprived mice, while in the normal mice haematopoiesis declined after day 28. The bone marrow contained both haemozoin and haemosiderin. The amount of pigment increased after day 7 in both normal and deprived mice.

2.4 Fluorescent antibody levels

(Fig. 2.8 and Table 2.8)

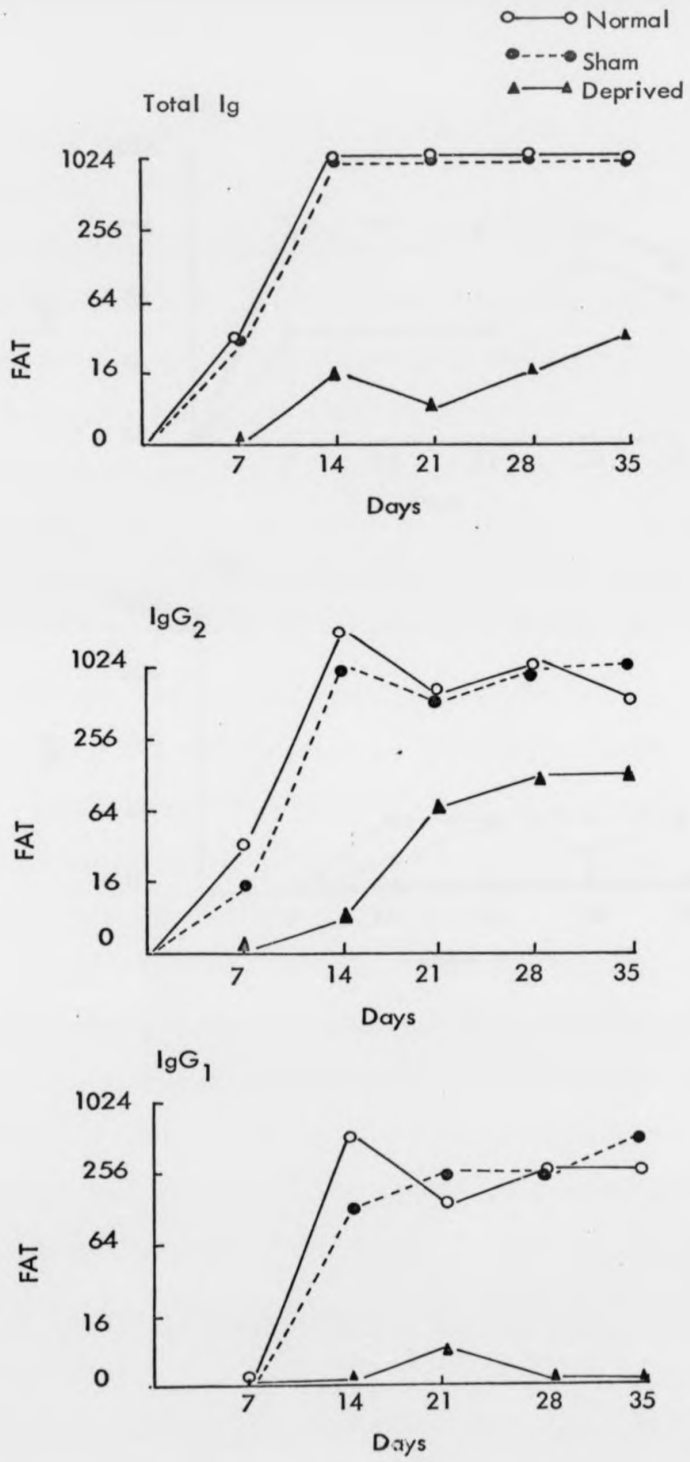
Using the indirect fluorescent antibody test (IFAT) anti-plasmodial antibody production was measured in normal, sham deprived, and deprived mice over a 35 day period. All antibody determinations were carried out on pooled serum samples.

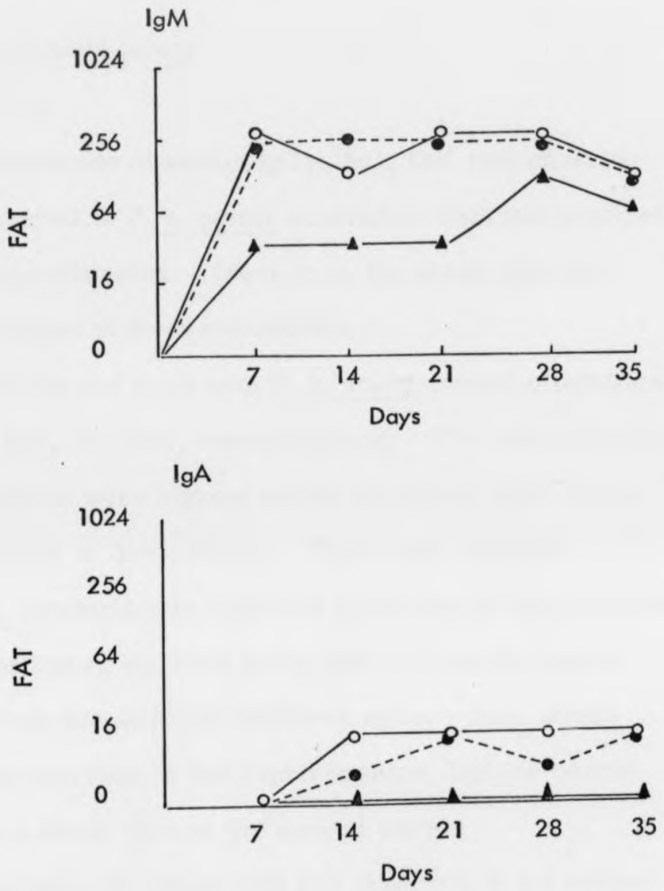
There was rapid production of antiplasmodial antibodies in the control (normal and sham) groups. High titres of IgG_1 and IgG_2 antibodies had built up by day 14 and considerably increased levels of these two sub-classes were maintained over the 35 day period of observation. In the deprived group, IgG antibody production was severely impaired. IgG_1 production was almost completely suppressed, very low levels being detected only on day 21 of infection. IgG_2 production was less severely affected but the titres recorded were substantially lower than in the control groups.

IgM was detected in both intact and deprived mice by day 7 of infection. In the control groups these levels were sustained. The level of IgM in the deprived group was lower than in the controls but IgM production was not impaired to the same extent as IgG. There was some increase in IgM production during the later stages of the infection in the deprived mice.

Very low levels of IgA were detected from day 14 onwards in the controls. IgA was not detected in the deprived mice.

Fig. 2.8. Fluorescent antibody levels (expressed as the reciprocal of the last dilution showing a + fluorescence) in normal, sham and T cell deprived mice infected with P.b. yoelii .


Normal
Sham
Deprived
Total Ig
1024
256
64
16
0
FAT
7
14
21
28
35
Days
IgG2
1024
256
64
16
0
FAT
7
14
21
28
35
Days
IgG1
1024
256
64
16
0
FAT
7
14
21
28
35
Days


IgM
1024
256
64
16
0
FAT
7
14
21
28
35
Days
IgA
1024
256
64
16
0
FAT
7
14
21
28
35
Days

2.5 Serum immunoglobulin levels

(Fig. 2.9 and Table 2.9)

The concentrations of serum IgG_1, IgG_2 and IgM immunoglobulins were measured in P. b. yoelii infected normal and deprived mice by radial immunodiffusion. (Sera from the sham deprived groups were not included in these estimations.)

Infection of normal mice with P. b. yoelii caused a significant increase in serum IgG_1 and IgG_2 concentrations. The concentrations of these two sub classes were highest on day 14, after which there was a gradual decrease in these levels. There was marked suppression of IgG_1 production in deprived mice, the levels recorded during the 35 day period of infection being only marginally higher than the resting levels in uninfected deprived mice. IgG_2 levels showed a significant increase in the deprived mice, but the concentrations were always lower than in the normal mice.

A rapid increase in serum IgM was observed in the normal group, peak levels being recorded by day 7. Substantially elevated levels of IgM were maintained until day 28 after which the concentration fell rapidly. The deprived mice also showed increased levels of IgM, maximum concentrations were recorded on day 14 after which there was a gradual fall in IgM levels.

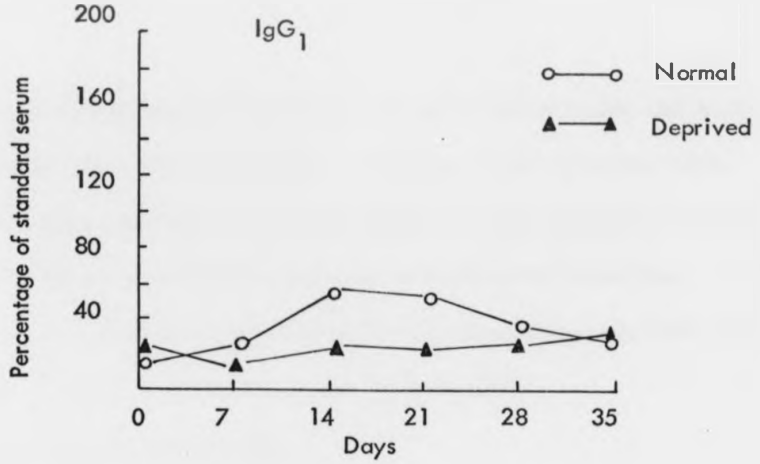
2.6 Steroid levels and the adrenal response

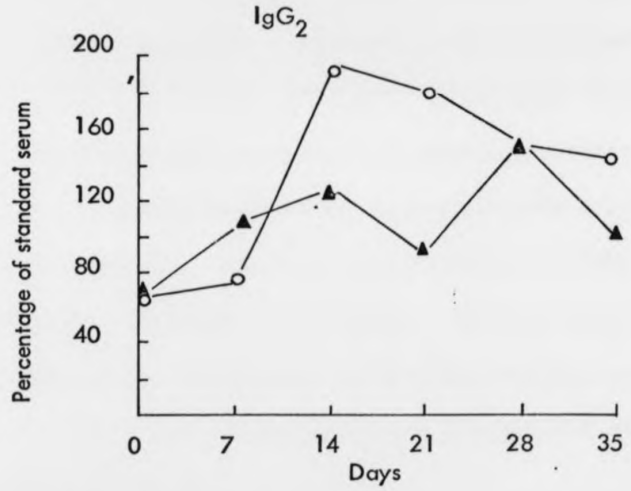
(Fig. 2.10 and Table 2.10)

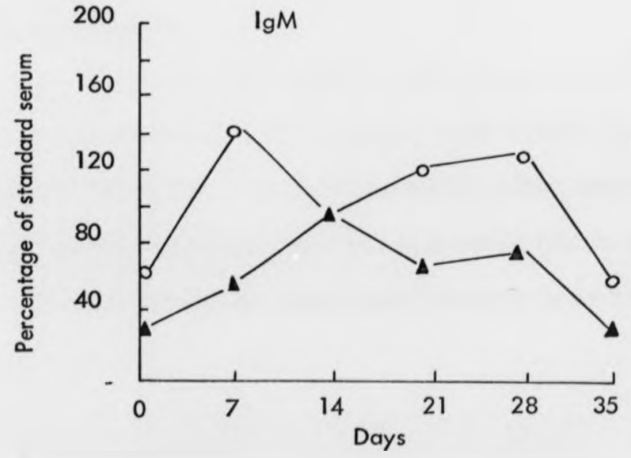
To determine whether P. b. yoelii infections caused any changes in steroid levels, a separate experiment was set up. A group of normal CBA mice was infected with 1×10^4 parasitized RBC,

Fig. 2.9 Serum immunoglobulin levels (expressed as a percentage of a standard serum) in normal and T cell deprived mice infected with P.b. yoelii .

Fig. 2.9 Serum immunoglobulin levels (expressed as a percentage of a standard serum) in normal and T cell deprived mice infected with P.b. yoelii .


IgG1
Normal
Deprived
Percentage of standard serum
200
160
120
80
40
0
7
14
21
28
35
Days


IgG2
Percentage of standard serum
200
160
120
80
40
0
7
14
21
28
35
Days


IgM
Percentage of standard serum
200
160
120
80
40
0
7
14
21
28
35
Days

and a group of uninfected controls was maintained under the same conditions as the infected group. Plasma corticosterone determinations were carried out on individual plasma samples collected from these mice at various intervals of time after infection. Adrenal and thymus weights were recorded at the same time, and the histopathology of these organs was also investigated.

Plasma corticosterone levels

Infected mice showed increased plasma corticosterone levels from day 9 onwards. Substantial differences between the infected and control groups were observed on days 14 and 16, at which times the infected group had plasma corticosterone concentrations of 14.5 and 19.8 µg/100 ml, compared with 7.7 and 9.0 µg/100 ml in the controls. (A peak parasitaemia of 4.80% was recorded on day 13 of this experiment.) 29 days after infection the mean plasma corticosterone level in the infected group had dropped to 7.80 µg/100 ml compared with 6.97 µg/100 ml in the control group at the same time.

The adrenal response

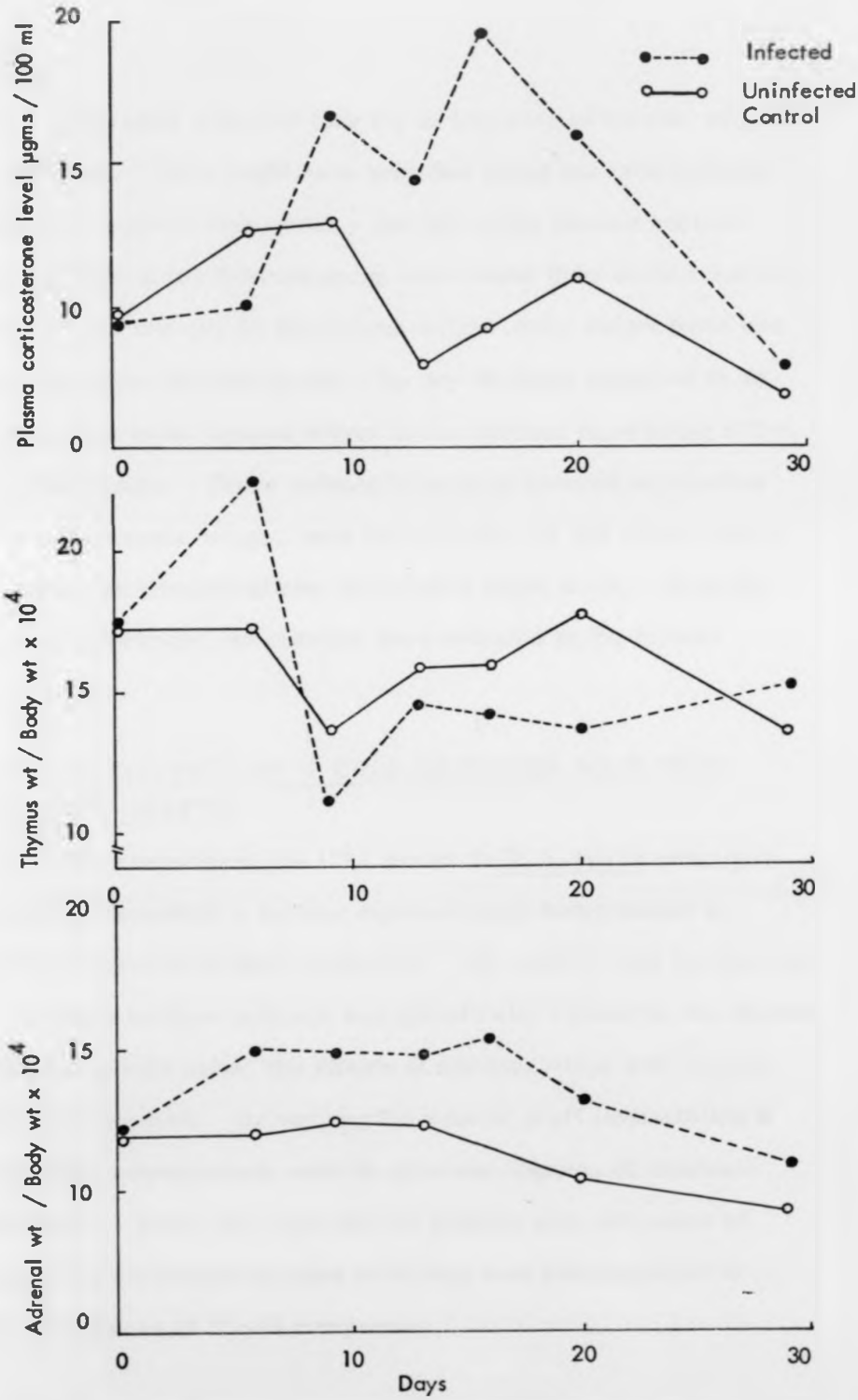
The adrenal weight : body weight ratios in the infected group were higher than in the controls: peak values were observed on day 16 (i.e. after the peak parasitaemia), after which, with the elimination of the infection there was a gradual fall in adrenal weights. Histologically no significant changes were seen in the adrenals.

Fig. 2.10 Plasma corticosterone levels, thymus weight/body weight ratio, and adrenal weight/body weight ratio in normal uninfected, and *P.b. yoelii* infected CBA mice.

Fig. 2.10a Concentration of plasma corticosterone.

Fig. 2.10b Thymus weight/body weight ratio.

Fig. 2.10c Adrenal weight/body weight ratio.


Infected
Uninfected Control
Plasma corticosterone level µgms / 100 ml
Thymus wt / Body wt x 10^4
Adrenal wt / Body wt x 10^4
Days
0
10
15
20
30

Thymus

Soon after infection (day 6), an increase in thymus weight was observed. This might have been due to the parasite initially exerting an adjuvant like effect. (At this stage plasma corticosterone levels in the infected group were lower than in the controls.) Between day 9 and day 20 the thymus weight : body weight ratio was depressed in the infected group. By day 29 there appeared to be recovery, the mean thymus weight in the infected mice being within the control range. There seemed to be good inverse correlation between the thymus weight : body weight ratio and the mean plasma corticosterone concentrations recorded in these mice. As in the previous experiment, no changes were detected in the thymus histologically.

3. RECONSTITUTION OF T CELL DEPRIVED MICE WITH THYMUS GRAFTS

The response of the CBA mouse to P. b. yoelii infections was thymus dependent - thymus deprived mice being unable to respond effectively to these infections. To confirm that the immunological lesion in these animals was specifically related to the absence of thymus derived cells, the effects of reconstitution with thymus grafts were studied. By varying the time of graft implantation it is possible to reconstitute mice to different degrees of immunocompetence. Using this approach an attempt was also made to compare the responses of mice which had been reconstituted to different degrees of T cell competence.

3.1 Experimental design

CBA/Lac mice were thymectomized at 6-8 weeks; 10 days later they were X-irradiated with 850 rads and reconstituted with 5×10^6 syngeneic bone marrow cells. These mice were divided into 3 groups A, B and C which were reconstituted with thymus grafts consisting of two thymus lobes from CBA/H.T6T6 neonates. All grafts were implanted under the kidney capsule.

Groups A, B and C were reconstituted with grafts on day -30, -15 and 0 in relation to the day of infection. The mice in group A were reconstituted 20 days after X-irradiation; hence, when the three groups of mice were infected 50 days had elapsed since irradiation. The infective inoculum consisted of 1×10^4 P. b. yoelii parasitized RBC. The mice in group C were reconstituted 2 hours after infection.

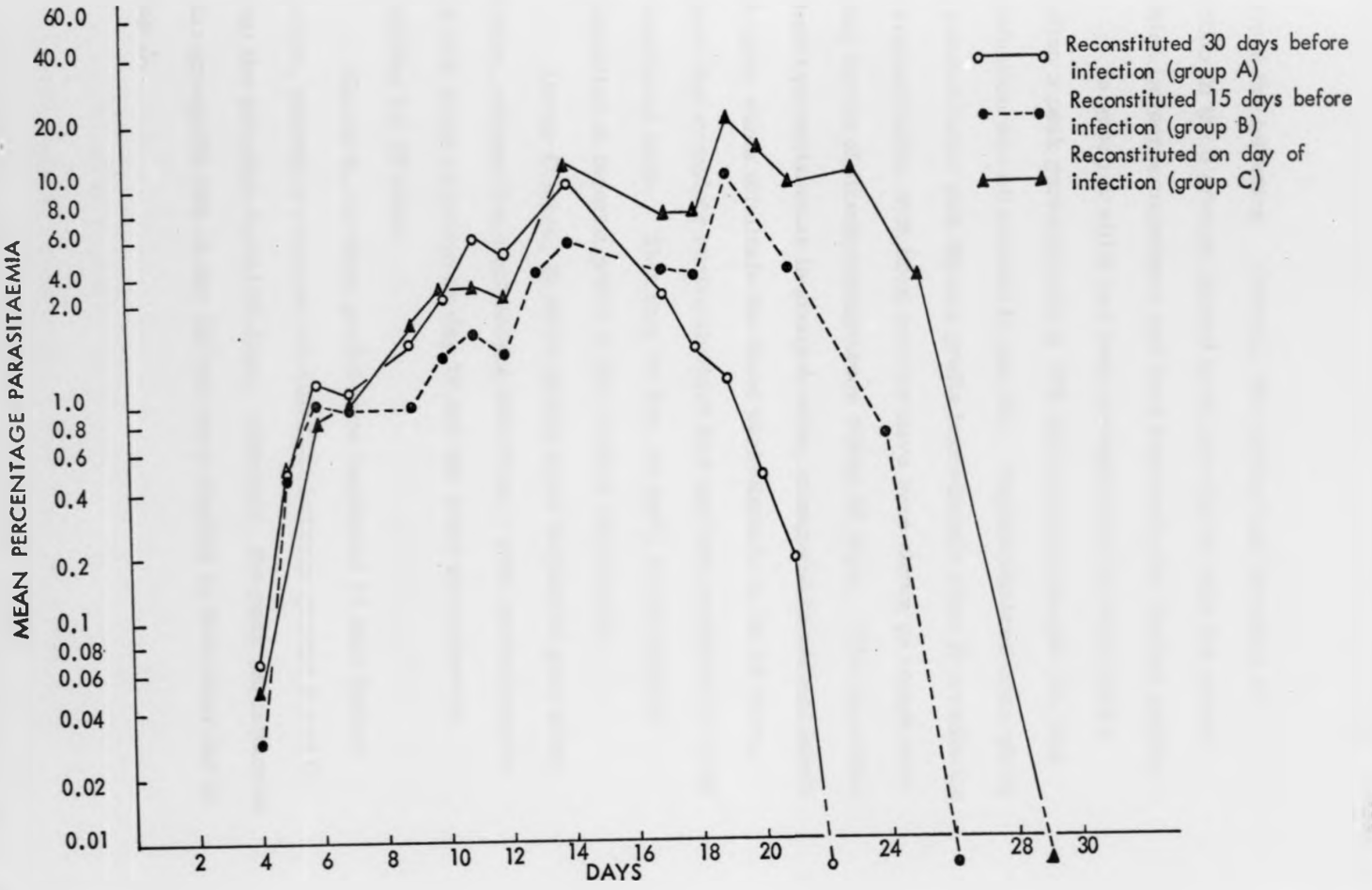
The parameters studied in this experiment were the blood parasitaemia and histopathological changes in the lymphoid organs. Groups of infected mice were sacrificed 7, 14, 21, 31 and 45 days after infection for histopathological studies. Some mice from groups A and B were also sacrificed for histopathology after graft implantation and before infection in order to follow the pattern of graft regeneration and immunological reconstitution.

3.2 Parasitaemias

(Fig. 2.11 and Table 2.11)

Unlike T cell deprived mice in which P. b. yoelii infections always proved fatal, all mice reconstituted with thymus grafts

Fig. 2.11 Mean percentage parasitaemias in P.b. yoelii infected T cell deprived mice reconstituted with thymus grafts 30 days before infection (group A), 15 days before infection (group B), or on the day of infection (group C).


MEAN PERCENTAGE PARASITAEMIA
60.0
40.0
20.0
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
24
28
30
DAYS
Reconstituted 30 days before infection (group A)
Reconstituted 15 days before infection (group B)
Reconstituted on day of infection (group C)

survived the infection. Overall, the pattern and duration of infection in the 3 groups showed good correlation with the extent to which immunocompetence had been restored with thymus grafts.

In group A which had been reconstituted 30 days before infection a peak parasitaemia of 10% was recorded on day 14, and the infection was eliminated by day 22. Thymectomized mice which are reconstituted with thymus grafts immediately after X-irradiation and reconstitution with bone marrow have been shown to regain near normal levels of immunocompetence within 30 days. The somewhat extended parasitaemias in group A mice, compared to normal intact CBA mice which eliminate the blood parasitaemia in 15-16 days, suggest that complete responsiveness had not been restored in these reconstituted mice. This may be due, in part, to the delayed implantation of thymus grafts in the present experiment.

Group C mice, in which grafts were implanted soon after infection, showed the most severe infections - peak parasitaemias of 23.86% being recorded on day 19 and the blood parasitaemia persisting for 29 days.

Group B, in which grafts were implanted 15 days before infection, showed a response intermediate between groups A and C, in that the infection lasted 26 days. However, the peak parasitaemia in this group (10.16% on day 19) was very similar to that observed in group A.

3.3 Immunopathology

The histopathological responses seen in the spleens of the 3 groups of reconstituted mice, formed a spectrum intermediate between that observed in the normal CBA mouse and the T cell deprived mouse. The cellular changes and the time course of the responses in group A mice were close to that observed in intact CBA mice, while the nature and pattern of responses in group C approached that seen in deprived mice. The state of the thymus grafts, as judged by weight and histological appearance, usually correlated with the degree of immunological responsiveness and in particular, with the amount of germinal centre activity seen. In group A, grafts examined during the infection were large and showed good cortico-medullary differentiation. In group C (which was grafted on the day of infection), the grafts initially involuted, and on day 7 consisted mainly of reticular cells. The grafts then regenerated and by day 31 normal thymic architecture had been restored, but the grafts still appeared smaller than control grafts implanted in normal uninfected mice. The germinal centre response was weakest in this group.

The reactive changes seen in the spleens and thymus grafts on day 21 of infection, were considered representative of the experiment as a whole, and will be described in some detail.

Group A.

(Graft implanted 30 days before infection)

The spleen was enlarged and the white pulp was more

extensive than the red pulp. The white pulp segments were prominent and consisted mainly of numerous large and active looking germinal centres arranged along the periphery of the follicles. The pyroninophilic cell response in the periarteriolar regions which had been pronounced earlier in the infection had receded, and was now limited to a small zone of cells cuffing the central arteriole. Red pulp activity was waning. The thymus grafts were large and showed good differentiation of both cortex and medulla.

Group B.

(Graft implanted 15 days before infection)

Splenomegaly was more pronounced than in group A and the red pulp was more extensive than the white pulp. The white pulp segments were smaller and appeared less active than in group A. The response in the 'thymus dependent' area was variable. In some follicles few pyroninophilic cells were observed; in others the zones were still quite extensive.

The thymus grafts were well differentiated and histologically showed no difference from group A grafts. However, the thymus graft weight : body weight ratio was 11.74 compared to 17.40 in group A.

Group C.

(Graft implanted 2 hours after infection)

The degree of splenomegaly was similar to that recorded in group B, but the red pulp was even more extensive. The white pulp segments were small, but well delineated. Germinal centre

development was poor and group C mice showed the least amount of germinal centre activity, at this stage of infection. Extensive zones of pyroninophilic cells, consisting of large indeterminate cells and some plasma cells were found surrounding the central arteriole. The overall histological picture was comparable to that seen in a P. b. yoelii infected deprived mouse 21 days after infection.

The thymus grafts were small and involuted, and consisted largely of cortical elements, little medullary tissue being present. The grafts were approximately 1/3 the size of a day 21 graft in an uninfected mouse and the thymus graft weight : body weight ratio was 4.75.

SECTION II. THE RESPONSE OF CBA MICE TO P. B. BERGHEI

1.1 Parasitaemias

(Fig. 2.12 and Table 2.12)

P. b. berghei produced a fulminant infection in the CBA mouse. With an inoculum of 1×10^4 parasitized RBC given I.P., parasites were detected in the peripheral blood within 2 to 3 days. The parasitaemia mounted rapidly; by day 10 the mean percentage parasitaemia was 4.75 and by day 18 it was 27.60. The infection proved fatal in all mice between day 20 and 26. The terminal parasitaemias ranged from 40 to 50%, and were significantly lower than the terminal parasitaemias seen in P. b. yoelii infected deprived mice.

1.2 Haematology

Packed cell volumes (PCV)

(Fig. 2.13 and Table 2.13)

P. b. berghei infected CBA mice developed a severe anaemia within two weeks of infection. The severity of the anaemia increased as the infection progressed, and by day 22 the haematocrit had fallen from 48.15% on day 0 to 11.08%. Mercado and Coatney (1951) who studied P. berghei infections in the mouse, also reported the development of a marked anaemia. More recently Hejna et al. (1974) found that in P. berghei infected CD-1 mice, transfusion of homologous erythrocytes could restore peripheral erythroid parameters to control levels and increase the survival rate of these mice significantly.

Fig. 2.12 Mean percentage parasitaemias in normal CBA mice infected with 1×10^4 P.b. berghei parasitized RBC.

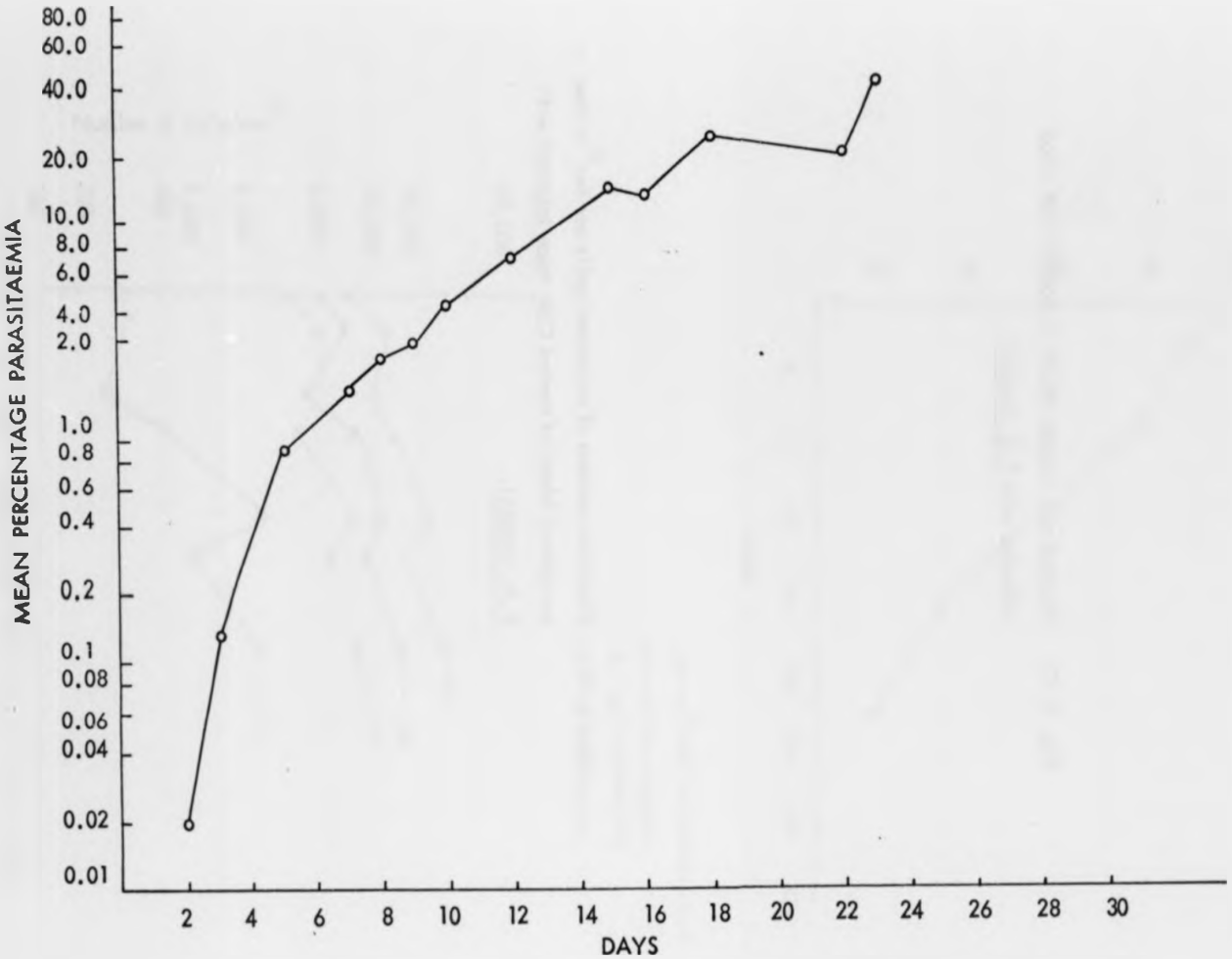
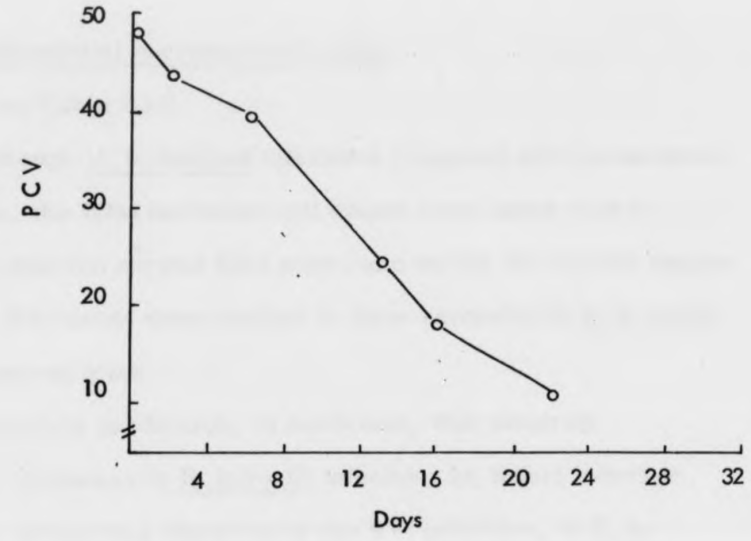
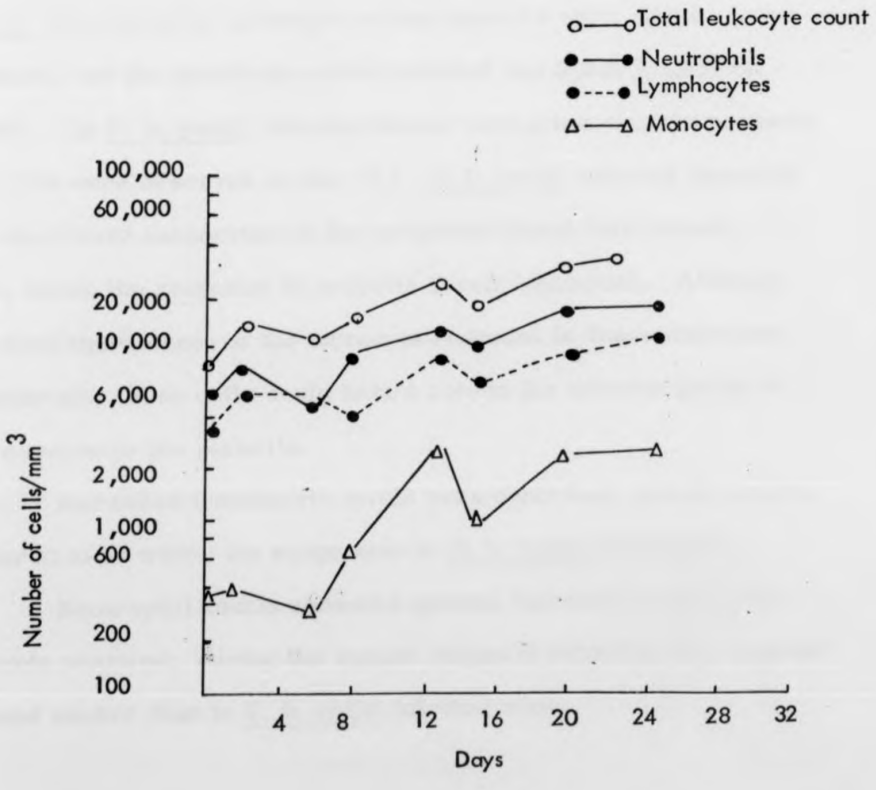

MEAN PERCENTAGE PARASITAEMIA
80.0
60.0
40.0
20.0
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS

Fig. 2.13 Packed cell volume (PCV) in normal CBA mice infected with P.b. berghei.

Fig. 2.14 Absolute numbers of nucleated cells per mm^3 in the peripheral blood of normal CBA mice infected with P.b. berghei.


50
40
30
20
10
PCV
4
8
12
16
20
24
28
32
Days


Total leukocyte count
Neutrophils
Lymphocytes
Monocytes
100,000
60,000
20,000
10,000
6,000
2,000
1,000
600
200
100
Number of cells/mm3
4
8
12
16
20
24
28
32
Days

Total and differential nucleated cell counts

(Fig. 2.14 and Table 2.14)

Although P. b. berghei infections triggered off a pronounced leukocytosis, the total nucleated cell counts were lower than in P. b. yoelii infected normal CBA mice, and during the earlier stages of infection the counts were similar to those recorded in P. b. yoelii infected deprived mice.

Monocyte production, in particular, was severely depressed. Whereas in P. b. yoelii infections an 8 fold increase in monocyte levels was observed by day 6 of infection, in P. b. berghei infected mice monocyte counts showed a very slight increase, and the maximum value recorded was 2,639 ± 1,654 on day 25. (In P. b. yoelii infected normal CBA mice maximum counts of 29,206 were observed on day 13.) P. b. yoelii infected deprived mice had fewer monocytes in the peripheral blood than normal mice, hence the response is probably T cell dependent. Although the exact significance of the monocyte response in these infections is uncertain, these cells could have a role in the effector phase of the response to the parasite.

Increased lymphocyte levels were observed, and the counts appeared to be within the range seen in P. b. yoelii infections.

Neutrophil counts showed a gradual increase as the parasitaemia mounted; during the earlier stages of infection the response seemed weaker than in P. b. yoelii infected mice.

1.3 Immunopathology

Histopathological changes in the spleen, axillary and mesenteric lymph nodes, Peyer's patches, thymus, liver and adrenals were studied on days 5, 9, 15, 18 and 22 of a P. b. berghei infection. As in the previous experiment body weights and organ weights of the tissues examined were recorded. Organ weights were not recorded on day 22.

A. Spleen

Spleen weight/ body weight ratio

(Fig. 2.15a and Table 2.15)

P. b. berghei induced marked splenomegaly. Changes in the spleen weight : body weight ratio paralleled the progressive parasitaemia. Within 9 days the spleen weight : body weight ratio had increased from 3.0 to 13.70 and by day 18 the value recorded was 24.40. The maximum splenomegaly observed was considerably less than in a P. b. yoelii infection in normal CBA mice. In these infections by day 14 the spleen weight : body weight ratio had reached 35.40.

Histopathology

Day 5

(Plate 2.14)

Within 5 days the spleen showed a slight increase in size. The white pulp was somewhat more extensive than the red pulp.

In the white pulp the follicles had increased in size. The germinal centres were distributed along the periphery of the follicles

and they contained some pyroninophilic blast cells; no pigment (haemosiderin or haemozoin) was seen in the germinal centres at this stage. In the periarteriolar regions, some large pyroninophilic cells had begun to accumulate. Morphologically, these cells resembled the indeterminate lymphoid cells which were seen around the central arteriole in P. b. yoelii infections.

The red pulp appeared haematopoietically active. It contained many large pyroninophilic blast cells and a few haemozoin containing macrophages.

Day 9

(Plate 2.15)

The splenomegaly was more pronounced and the red pulp was more extensive than the white pulp.

The white pulp segments were well delineated and the germinal centres showed essentially the same constituents as at day 5. Haemosiderin was observed in some macrophages. An extensive zone of pyroninophilic cells was now present around the central arteriole. This included a few mature plasma cells.

The red pulp was very active looking and contained large numbers of heavily pyroninophilic cells, including mature plasma cells. These pyroninophilic cells appeared to be concentrated along the peripheral regions of the red pulp. A few megakaryocyte-like cells and many haemozoin containing macrophages were also present.

Day 12 to Day 15

(Plates 2.16 and 2.17)

By day 15 the red pulp was much more extensive than the white pulp and appeared to dominate the histopathological picture of the spleen.

The white pulp regions appeared smaller than at day 9 and were not so well defined. The germinal centres were arranged along the periphery of the mantle and contained many large pyroninophilic cells and some haemosiderin within macrophages. They appeared to be less well developed than in P. b. yoelii infections at a comparable stage. Some megakaryocytes were seen in the white pulp. These cells are normally present only in the red pulp; their presence in the white pulp was probably indicative of the extent to which the normal splenic architecture had been disrupted. There was further expansion of the pyroninophilic cell population in the 'thymus dependent' periarteriolar region. Few mature plasma cells were observed.

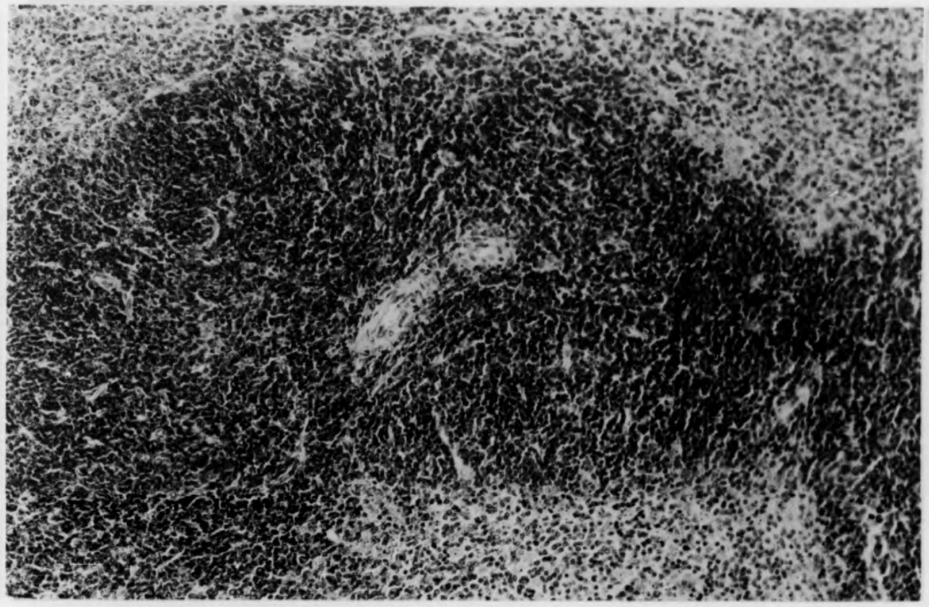
The red pulp was active and showed massive hyperplasia. It was infiltrated with pyroninophilic cells and haemozoin containing macrophages. The pyroninophilic cells and the haemozoin pigment were distributed throughout the red pulp. Most of the pyroninophilic cells were large and included mature and immature plasma cells.

Day 18

By day 18, the splenomegaly was even more severe. The reactive changes in the red and white pulp showed little change from

Plate 2.14 Normal CBA mouse; day 5 P. b. berghei infection. White pulp of spleen showing an accumulation of pyroninophilic cells around the central arteriole. M.G.P. x 196

Plate 2.15 Normal CBA mouse; day 9 P. b. berghei infection. An extensive zone of pyroninophilic cells is found surrounding the central arteriole. Germinal centres are present along the peripheral regions of the white pulp. M.G.P. x 196



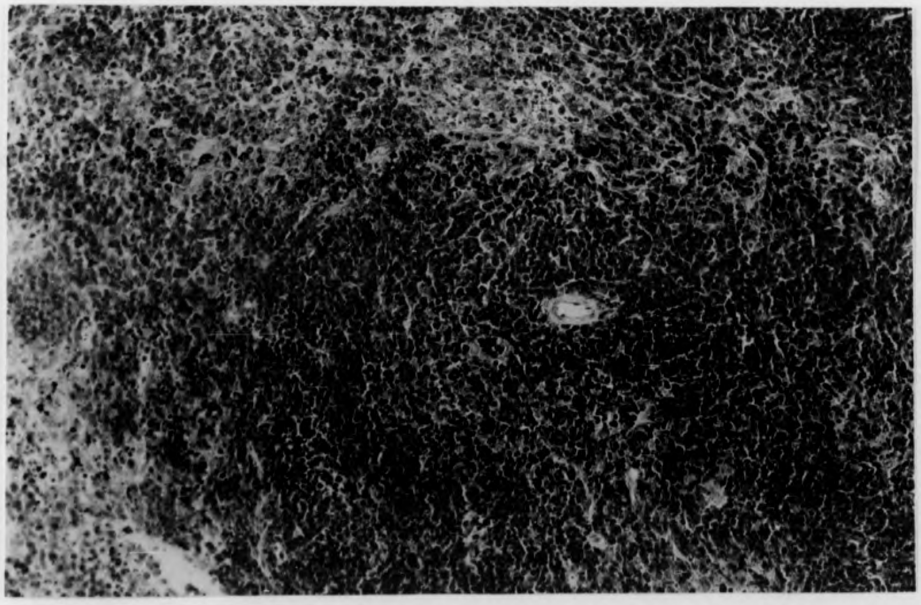
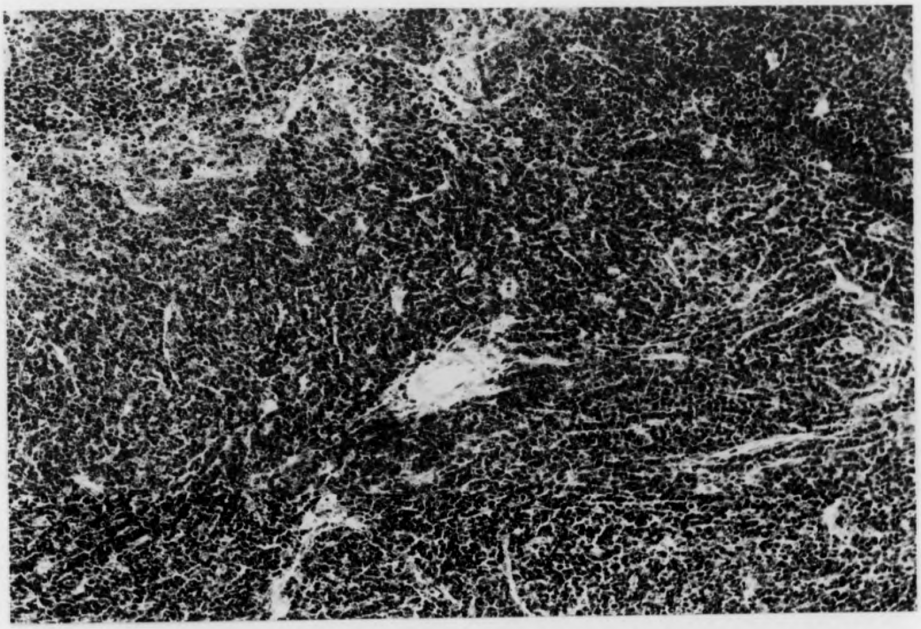
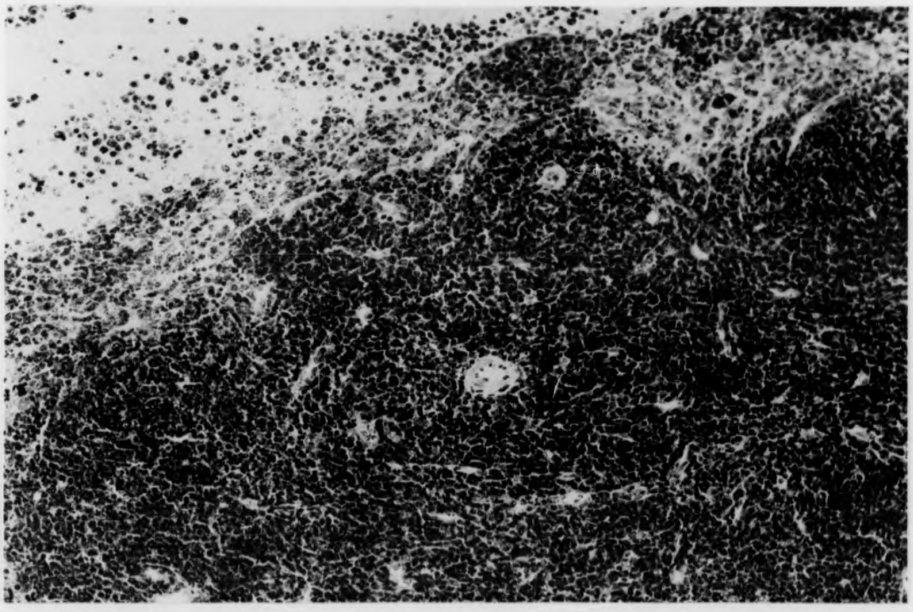


Plate 2.16 Normal CBA mouse; day 12 P. b. berghei infection. The white pulp segments of the spleen are not clearly delineated; the dominant feature of the follicle is the intense pyroninophilia around the central arteriole. Germinal centres are poorly defined. M.G.P. x 196

Plate 2.17 Normal CBA mouse; day 15 P.b. berghei infection. Spleen showing further expansion of the zone of pyroninophilic cells which surrounds the central arteriole. M.G.P. x 196





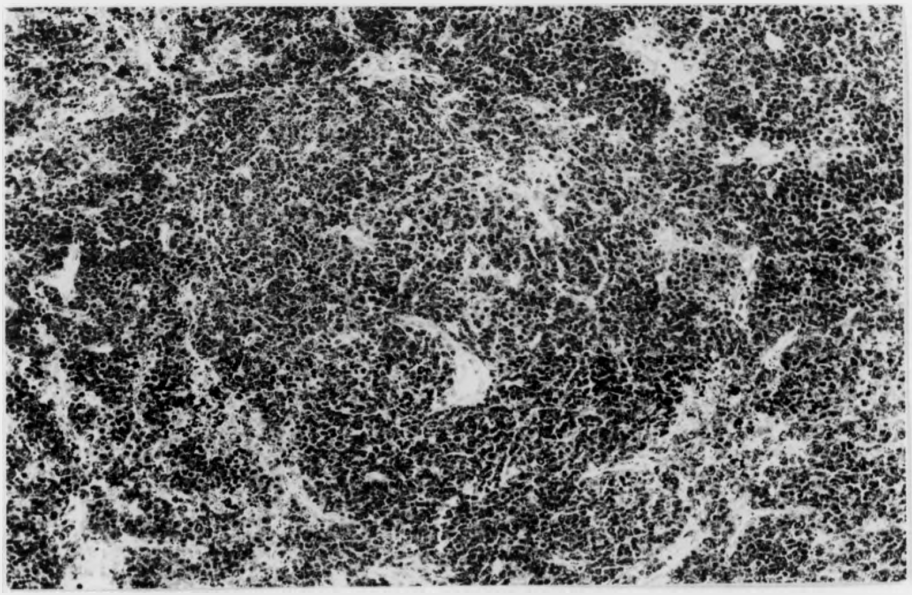


Plate 2.18 Normal CBA mouse; day 24 P. b. berghei infection. Spleen showing poorly demarcated white pulp segments surrounded by hyperplastic red pulp. Germinal centres are present but do not look very active; the pyroninophilic cell response in the periarteriolar region is maximal. Macrophages containing large clumps of haemozoin are present in the red pulp. M.G.P. x 196

that seen on day 15. The white pulp regions were dominated by massive zones of pyroninophilic cells cuffing the central arterioles. The intensity of this response appeared to parallel the pattern of infection very closely.

Day 24

(Plate 2.18)

There was little change in spleen size between day 18 and day 24 of infection. Red pulp activity appeared to be waning and as a result the white pulp segments were better defined.

The white pulp consisted mainly of large zones of pyroninophilic cells in the periarteriolar regions. Germinal centres were small and not very active looking. They were $\frac{1}{2}$ to $\frac{1}{3}$ the size of the germinal centres seen in P. b. yoelii infections in intact CBA mice at a similar stage of infection. The number of germinal centres was also much reduced compared to P.b. yoelii infections.

Red pulp activity was declining, but many pyroninophilic cells and haemozoin containing macrophages were present.

B. Lymph Nodes

(Fig. 2.15b and Table 2.15)

P. b. berghei infected CBA mice showed increased axillary lymph node weight : body weight ratios. Maximum values were recorded on day 15 of infection. Histologically no significant changes were observed in the axillary or mesenteric lymph nodes of infected mice.

C. Peyer's Patches

No reactive changes were detected during the 24 day period of study.

D. Thymus

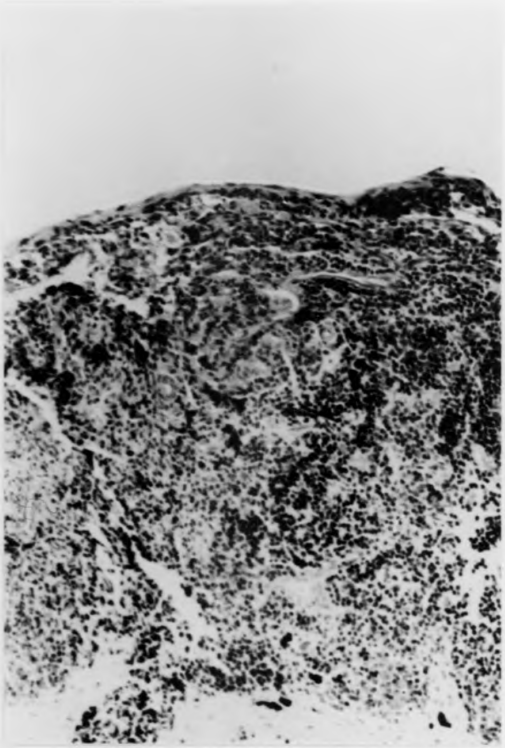
(Fig. 2.15c, Table 2.15 and Plates 2.19 to 2.22)

P. b. berghei infections caused gradual involution of the thymus. Within 5 days of infection, a drop in the thymus weight : body weight ratio was detected and this trend continued as the infection progressed. By day 18 the thymus weight : body weight ratio had dropped to 1.58, from a value of 8.00 on day 0.

Histologically, the thymus showed a characteristic series of changes as the infection progressed. During the early stages of infection, no reactive changes were seen. On day 9 there appeared to be some evidence of cortical depletion, but this was restricted to slight denudation of the thymic cortex. By day 15, considerable depletion of the cortex had occurred. Hassall's corpuscles, which consist of concentrically arranged epithelial cells, were a prominent feature of the thymic medulla at this stage; many of these corpuscles contained pigment within macrophages. There was also some germinal centre development within the medulla, and accumulations of pyroninophilic cells including a few plasma cells, were observed along the peripheral regions of the thymus. From day 18 onwards, in addition to severe destruction of the thymic cortex, there appeared to be considerable medullary depletion as well. In the terminal stages of the infection, the thymus

Plate 2.19 Thymus of normal uninfected CBA mouse. Note the relative proportions of cortex and medulla. Haematoxylin and Eosin (H.& E.) x 196

Plate 2.20 Thymus of CBA mouse; day 18 P. b. berghei infection, showing extensive depletion of the thymic cortex. H.& E. x 196



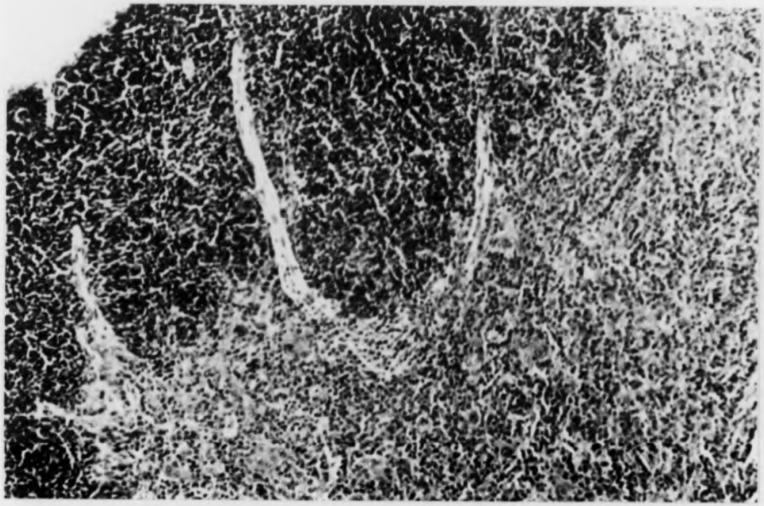
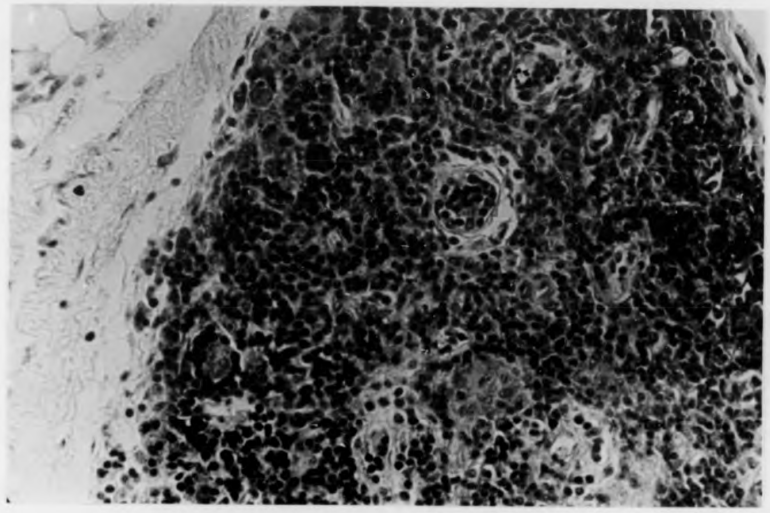
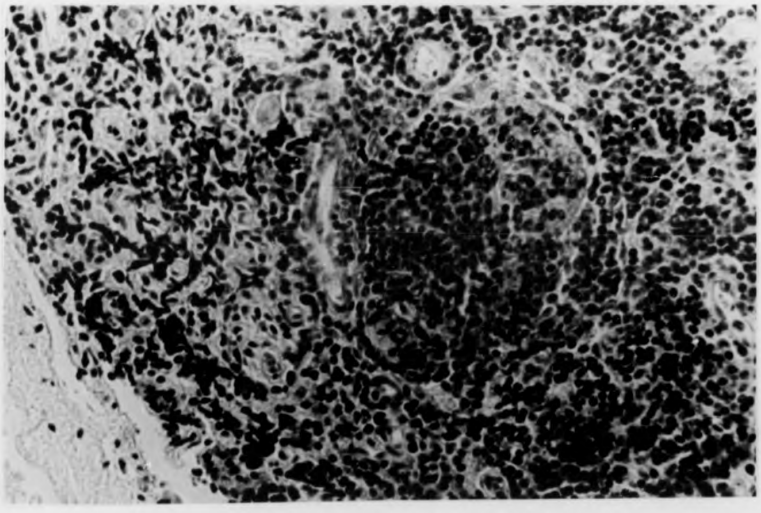
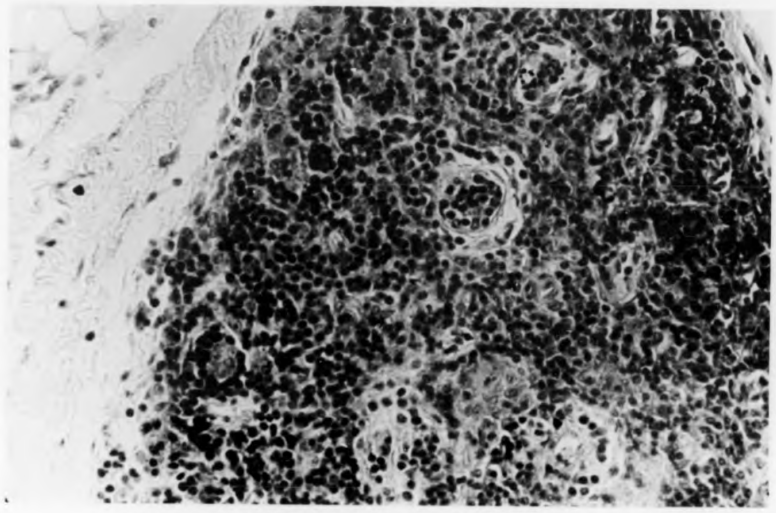


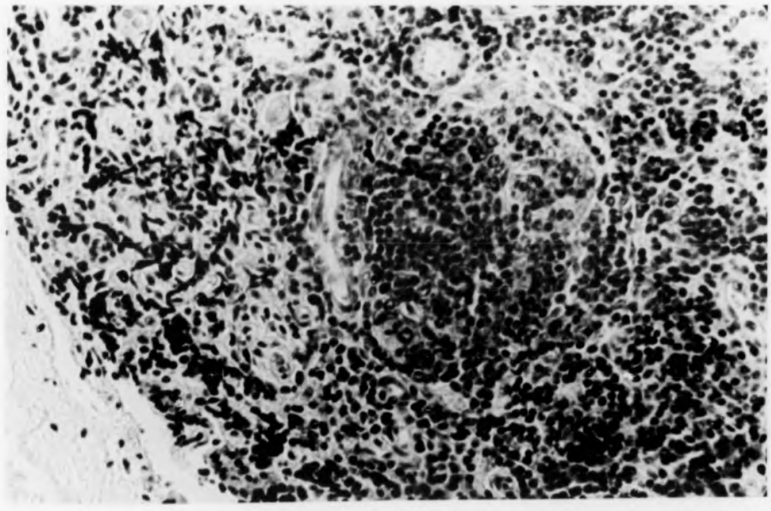
Plate 2.21 Thymus of CBA mouse; day 15 P. b. berghei infection, showing Hassall's corpuscles. H. & E. x400

Plate 2.22 Thymus of CBA mouse; day 15 P. b. berghei infection, showing germinal centre development. H. & E. x400









presented a grossly atrophied appearance characteristically found in states of acute stress or illness.

E. Adrenals

(Fig. 2.15d and Table 2.15)

Adrenal weights and the adrenal weight : body weight ratios were increased during the infection.

Significant adrenal hypertrophy, particularly of the adrenal cortex was detected from day 9 onwards. The degree of adrenal cortical activity appeared to increase as the parasitaemia mounted. Marked expansion of all three zones of the adrenal cortex was seen from day 15 onwards.

F. Liver

Haemozoin pigment was seen in the Kupffer cells of the liver from day 9 onwards, and the amount of pigment increased progressively. Foci of necrosis and accumulations of pyronino-philic cells, including many blast cells were observed at day 15, and these features became more intense as the infection progressed.

1.4 Steroid levels

Plasma corticosterone determinations were carried out on pooled plasma samples obtained from mice on day 15 of a P. b. berghei infection, and from a group of normal uninfected mice. The mean concentration of corticosterone in the plasma of the infected group was 17.5 µg/100 ml compared to 10.0 µg/100 ml in the controls.

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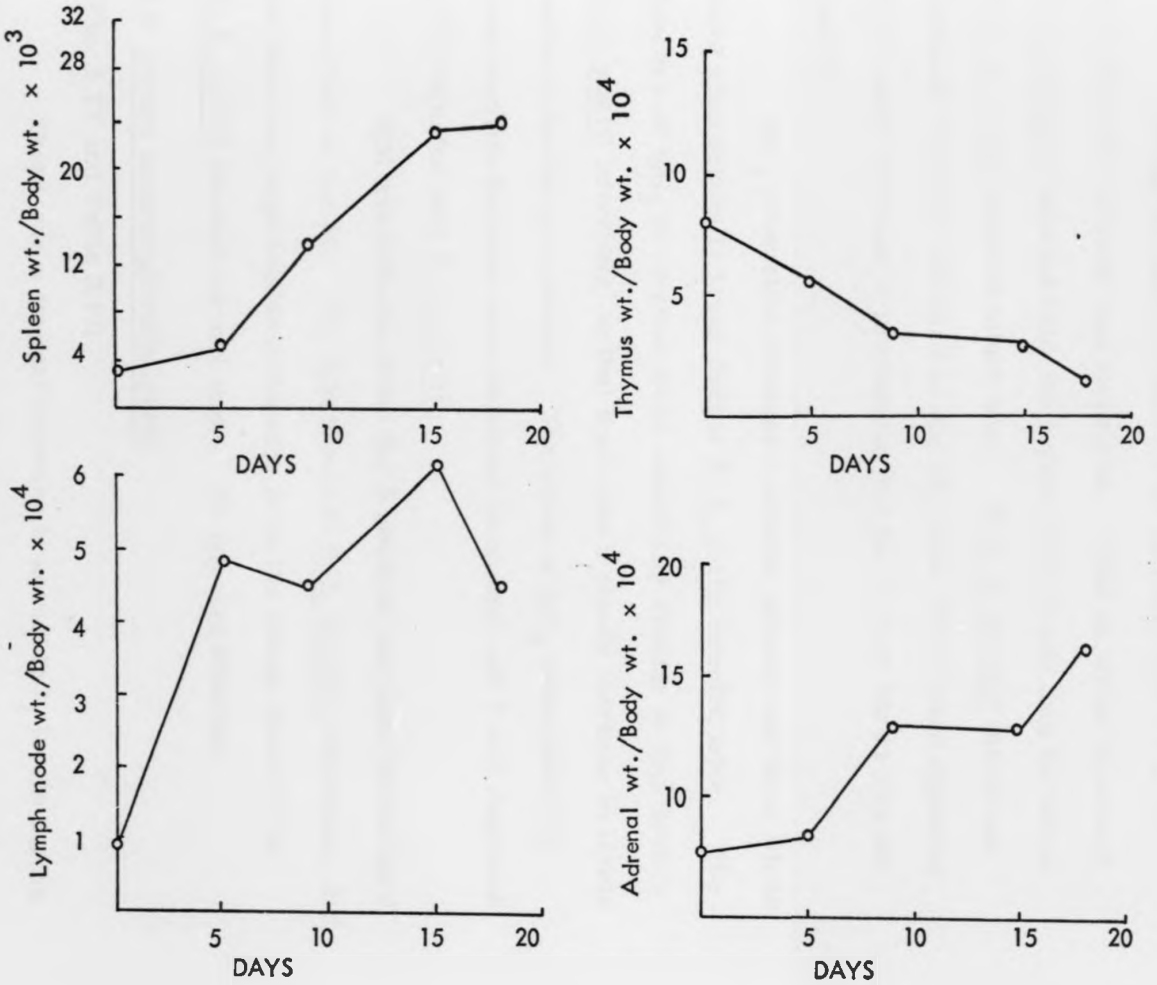
Fig. 2.15 Organ weight/body weight ratios in normal CBA mice infected with P.b. berghei.

Fig. 2.15a Spleen weight/body weight ratio.

Fig. 2.15b Axillary lymph node weight/body weight ratio.

Fig. 2.15c Thymus weight/body weight ratio.

Fig. 2.15d Adrenal weight/body weight ratio.


Spleen wt./Body wt. × 10^3
32
28
20
12
4
5
10
15
20
DAYS
Thymus wt./Body wt. × 10^4
15
10
5
5
10
15
20
DAYS
Lymph node wt./Body wt. × 10^4
6
5
4
3
2
1
5
10
15
20
DAYS
Adrenal wt./Body wt. × 10^4
20
15
10
5
10
15
20
DAYS

1.5 Fluorescent antibody levels

(Fig. 2.16 and Table 2.16)

Using the indirect fluorescent antibody test (I.F.A.T.) anti parasite antibody was measured. Total Ig levels in normal P. b. berghei infected CBA mice were much lower than the titres in P. b. yoelii infected intact mice. In P. b. berghei infections antibody was first detected on day 10, after which there appeared to be some increase in production, but the highest titre recorded was 64.

IgG_1 production followed a similar pattern and these titres were also generally lower than in P. b. yoelii infected mice. The kinetics of IgG_2 production were essentially similar to that seen in P. b. yoelii infections, in that there was a steady increase in levels as the infection progressed. The titres of IgG_2 were generally intermediate between those observed in normal and T cell deprived mice infected with P. b. yoelii.

IgM was detected from day 5 onwards and peak titres were recorded on day 15. The IgM levels in P. b. berghei infections, did not show any appreciable difference from the levels observed in P. b. yoelii infected normal mice. No IgA was detected.

1.6 Serum immunoglobulin levels

(Fig. 2.17 and Table 2.17)

There was a gradual increase in serum IgG_1 levels during the course of infection. Over the first 16 days the concentration

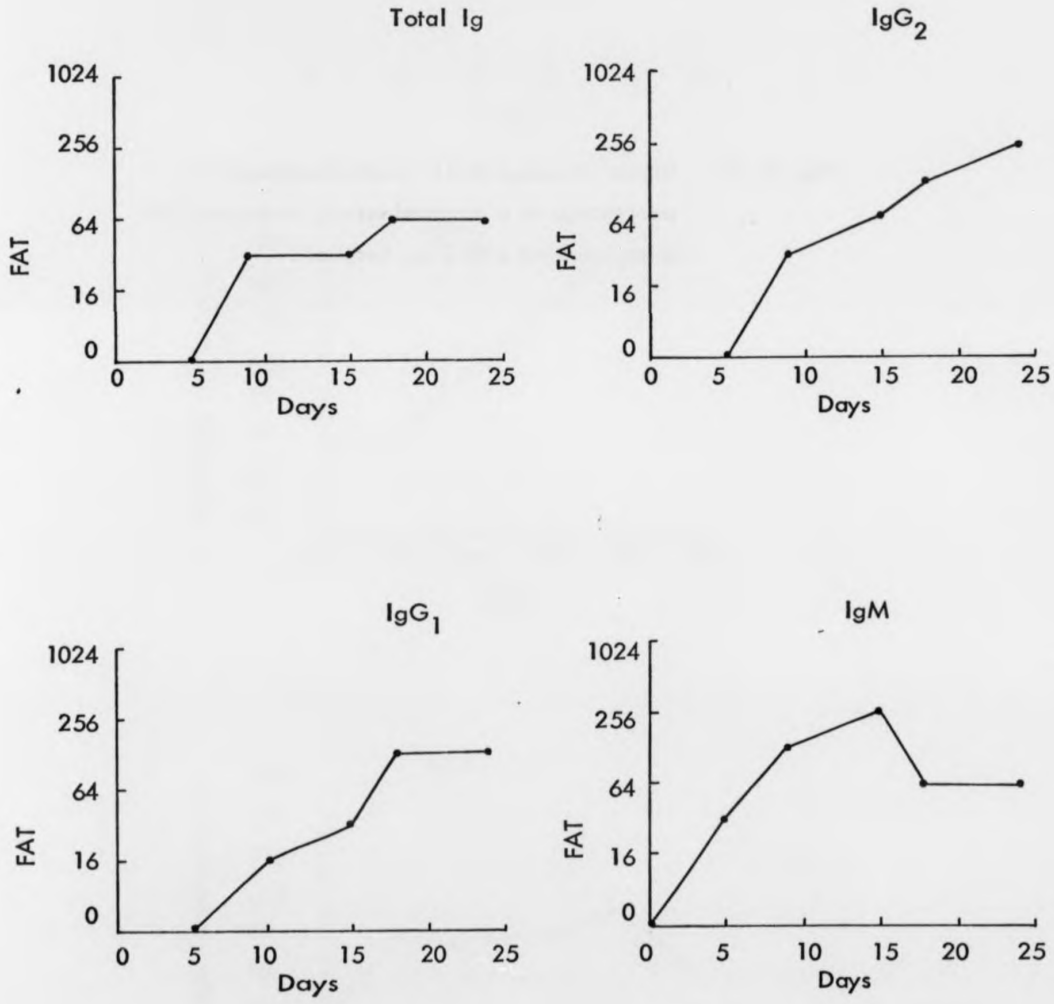

Total Ig
IgG2
IgG1
IgM
FAT
1024
256
64
16
0
0
5
10
15
20
25
Days

Fig. 2.16 Fluorescent antibody levels (expressed as the reciprocal of the last dilution showing a + fluorescence) in normal CBA mice infected with P.b. berghei.

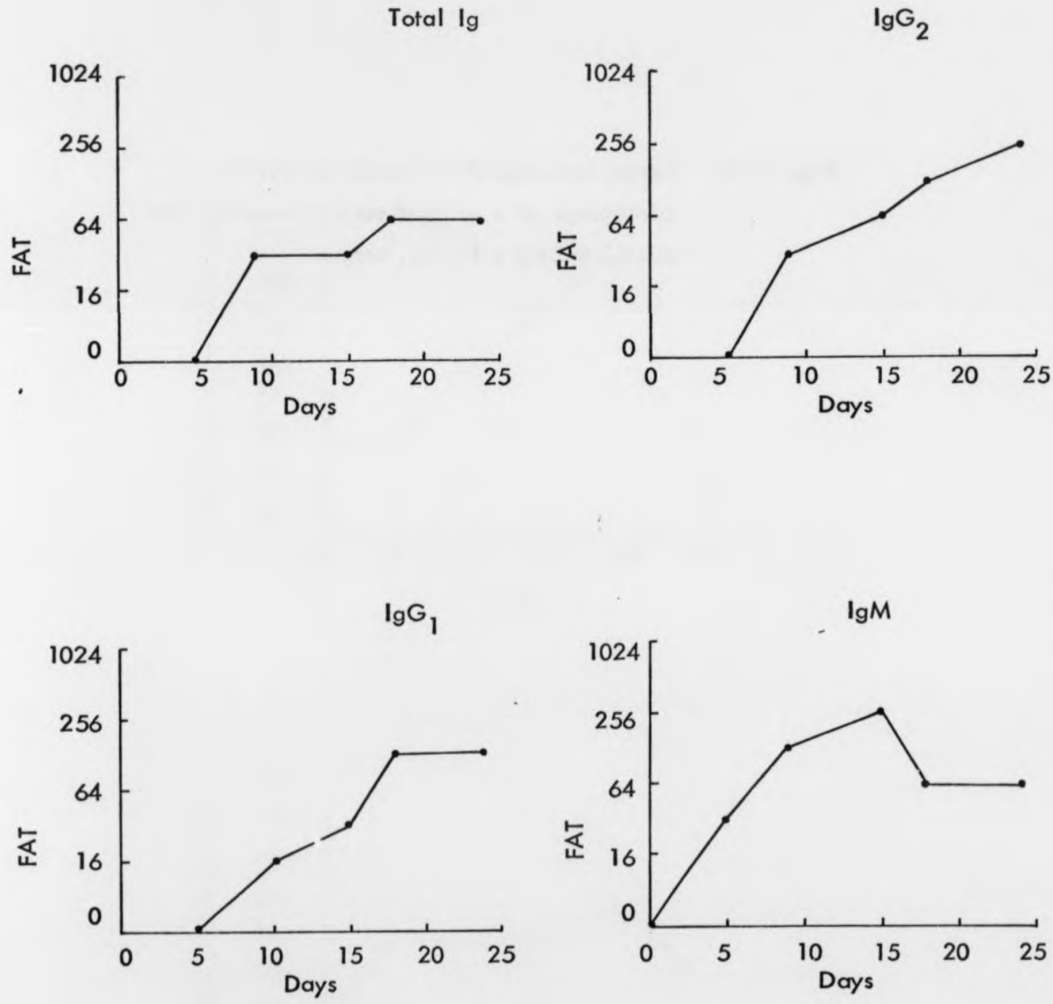
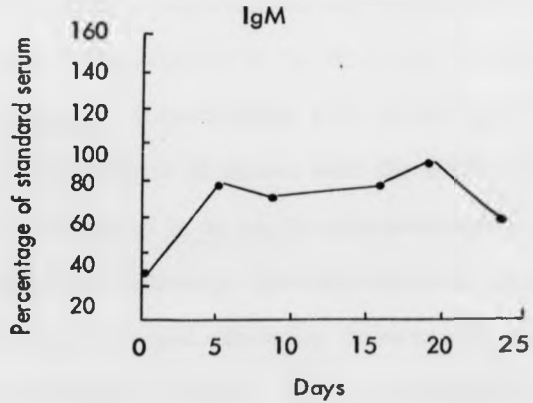
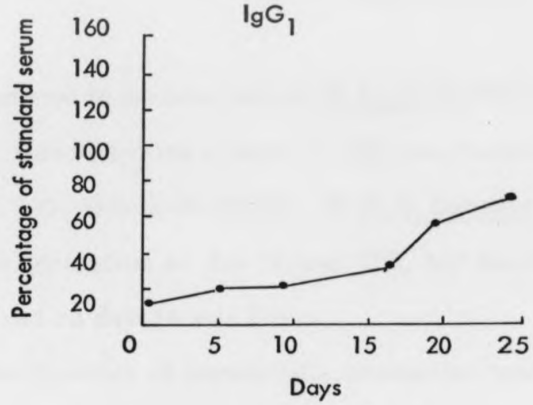
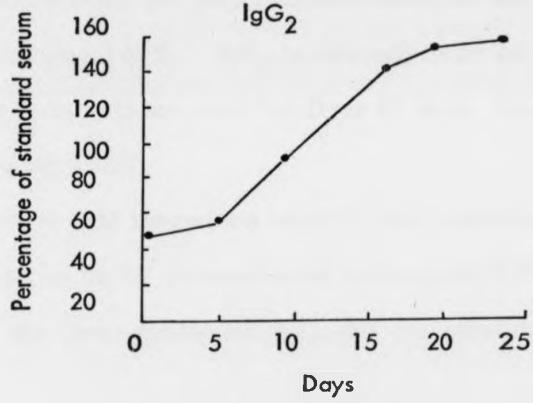

Total Ig
IgG2
IgG1
IgM
FAT
1024
256
64
16
0
0
5
10
15
20
25
Days

Fig. 2.17 Serum immunoglobulin levels (expressed as a percentage of a standard serum) in normal CBA mice infected with P.b. berghei.


IgM
Percentage of standard serum
160
140
120
100
80
60
40
20
0
5
10
15
20
25
Days


IgG1
Percentage of standard serum
160
140
120
100
80
60
40
20
0
5
10
15
20
25
Days


IgG2
Percentage of standard serum
160
140
120
100
80
60
40
20
0
5
10
15
20
25
Days

of IgG_1 appeared to be less than in P. b. yoelii infections. In P. b. yoelii infected mice a level of 55% was observed on day 14, after which IgG_1 levels declined. In P. b. berghei infected mice the IgG_1 concentration on day 16 was 31%, but the level rose thereafter and on day 34 was 70%.

The kinetics of serum IgG_2 production were similar to that detected in the fluorescent antibody test. IgG_2 levels showed a steady increase as the parasitaemia mounted and by day 16 the concentration was 140%. IgG_2 levels appeared to be slightly higher in P. b. yoelii infections over the first 14 days, the concentration on day 14 being 195%.

Serum IgM increased rapidly after infection, IgM levels increasing from 25.5% in uninfected controls to 77% by day 5. Thereafter the level plateaued until day 19, after which it decreased.

SECTION III. THE RESPONSE OF T.O. MICE TO P.B.YOELII AND P.B.BERGHEI

Preliminary experiments had shown that outbred T.O. Swiss mice were more susceptible to the malarial parasites P. b. yoelii and P. b. berghei. Experiments were carried out to compare the responses of this strain of mouse with the inbred CBA strain.

In the case of P. b. yoelii infections the parameters studied were the blood parasitaemia, histopathological changes in the spleen, lymph nodes, thymus and adrenals; fluorescent antibody levels and plasma corticosterone levels. In P. b. berghei infections, investigations

were limited to a study of the blood parasitaemia, histopathology of the spleen, thymus and adrenals, total and differential nucleated cell counts on the peripheral blood, and fluorescent antibody levels.

1. THE RESPONSE OF T.O. MICE TO P.B. YOELII

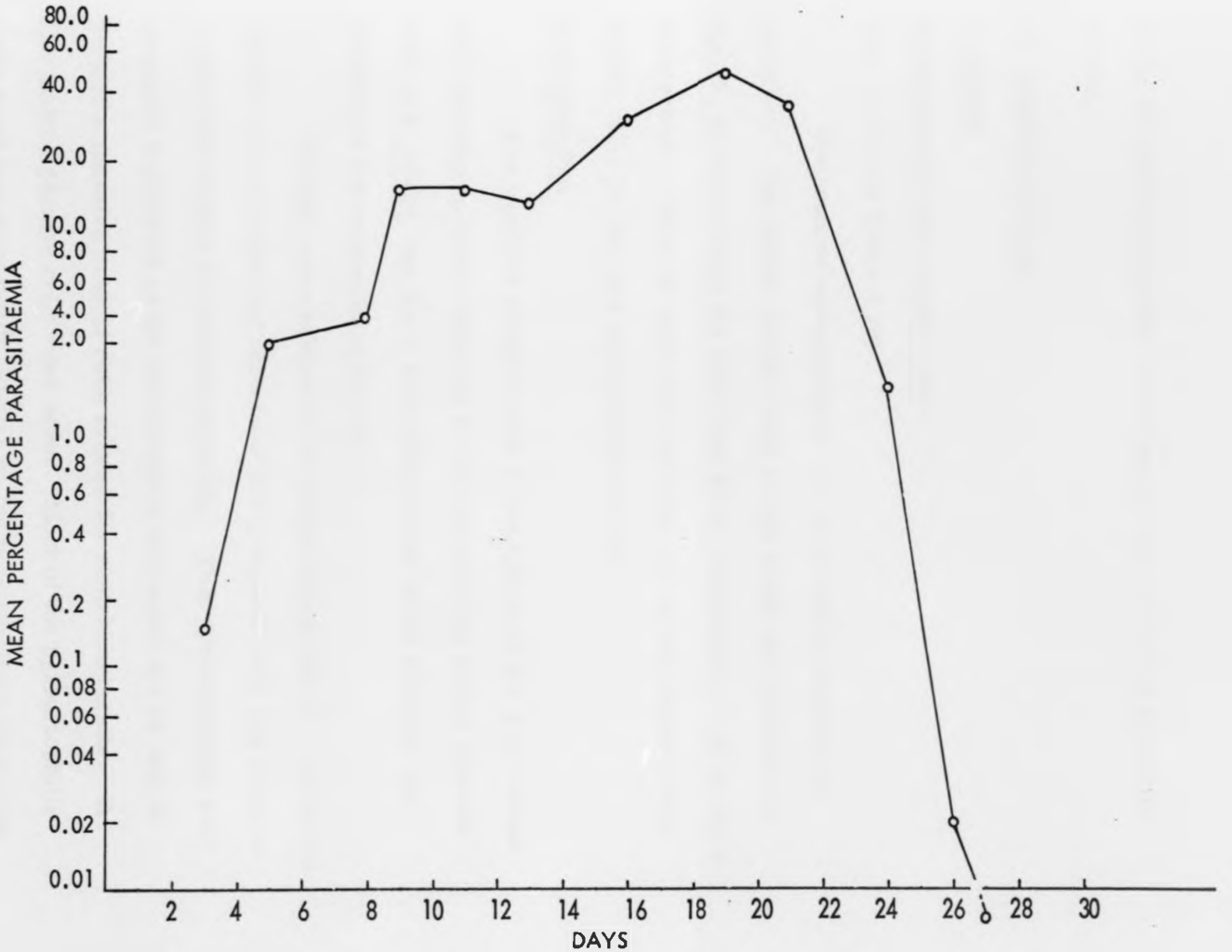
1.1 Parasitaemias

(Fig. 2.18 and Table 2.18)

P. b. yoelii infections were studied in a group of T.O. mice aged 10 to 12 weeks, which were infected with an inoculum consisting of 1×10^4 parasitized RBC I.P.. The resulting infection was much more severe than in the CBA mouse. Parasites were seen in the peripheral blood by day 3, the parasitaemia increased steadily and by day 11 16.47% of the RBC were parasitized. Unlike in the CBA mouse in which there was rapid regression of the parasitaemia from around day 10 to 12, and clearance of the blood parasitaemia by day 16, in the T.O. mouse the parasitaemia increased further and on day 16 the mean percentage parasitaemia was 34.92%. Between day 18 and day 23 the infection killed 45% of the mice. Maximum parasitaemias, in the group as a whole, were recorded on day 19 (56.30%) after which there was gradual regression of the infection; all mice which survived the infection were aparasitaemic by day 27.

The age of the T.O. mice seems to be an important factor in determining the outcome of a P. b. yoelii infection. Experiments not reported here, have shown that with younger mice, aged 6 to 7

Fig. 2.18 Mean percentage parasitaemias in outbred T.O. mice infected with 1×10^4 P.b. yoelii parasitized RBC.


MEAN PERCENTAGE PARASITAEMIA
80.0
60.0
40.0
20.0
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS

weeks the infection is often more severe and increased mortality is seen.

1.2 Immunopathology

A. Spleen

Spleen weight/body weight ratio

(Fig. 2.19a and Table 2.19)

Pronounced splenomegaly was observed throughout the infection. The spleen weight:body weight ratio was maximal on day 17, at which stage the ratio was 36.69 compared to 5.80 on day 0 of infection. Even 59 days after infection the spleen weight:body weight ratio (11.69) was significantly raised.

Histopathology

The reactive changes seen in the spleen of the T.O. mouse were similar to those observed in the normal CBA mouse infected with P. b. yoelii, but there were differences in the intensity and kinetics of the responses observed.

Changes were evident in the white pulp by day 6. Germinal centres had enlarged and pyroninophilic lymphoid cells had begun to accumulate around the central arteriole. This pyroninophilic cell response intensified as the parasitaemia increased and by day 14 formed a massive zone of cells around the central arteriole. A few mature plasma cells were seen. Most of the pyroninophilic cells were morphologically similar to the indeterminate blast type cells seen in the CBA mouse. This response was maximal at the peak of the infection (day 19). In those mice which survived the

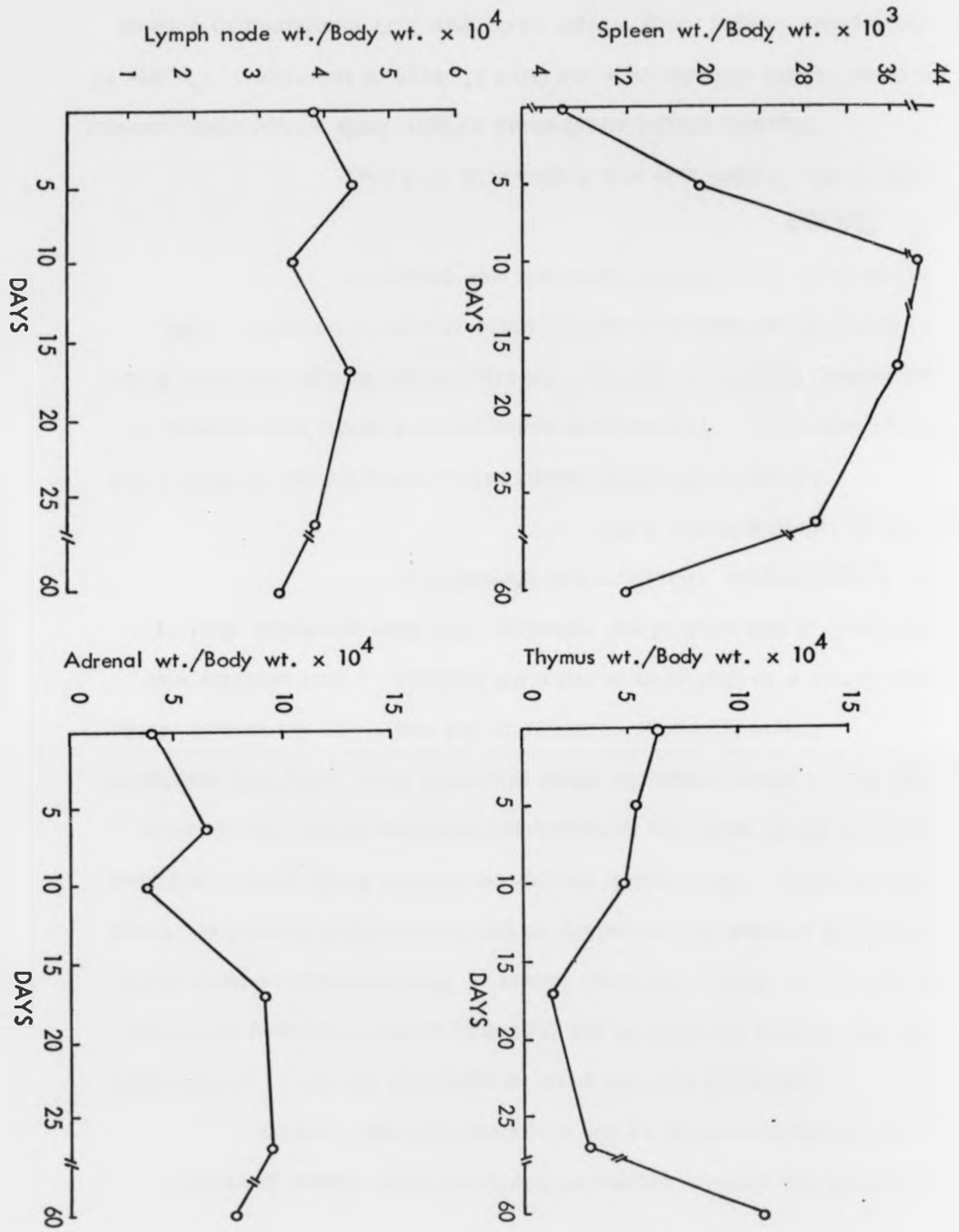
Fig. 2.19 Organ weight/body weight ratio in T.O. mice infected with P.b. yoelii

Fig. 2.19a Spleen weight/body weight ratio

Fig. 2.19b Axillary lymph node weight/body weight ratio

Fig. 2.19c Thymus weight/body weight ratio

Fig. 2.19d Adrenal weight/body weight ratio.



infection the thymus dependent periarteriolar region gradually returned to normality as the parasitaemia was cleared.

Germinal centres were conspicuous by day 14 of infection, but the overall kinetics of the germinal centre response appeared to be slower than in the CBA mouse. The germinal centres were arranged around the periphery of the follicles and showed the usual constituents. Mice which recovered showed large active germinal centres which were the predominant features of the spleens after day 30, at which stage the other elements were relatively quiescent.

There was much activity in the red pulp, the nature of which was similar to that seen in the CBA spleens. This activity was maximal at the peak of the infection, and then gradually declined.

B. Lymph Nodes (Axillary and Mesenteric)

(Fig. 2.19b and Table 2.19)

Lymph node : body weight ratios were raised on days 5 and 17 of infection. The axillary nodes showed some enlargement of germinal centres on day 14. No significant change was seen in the axillary or mesenteric nodes at other stages of infection. No evidence of paracortical depletion was observed.

C. Thymus

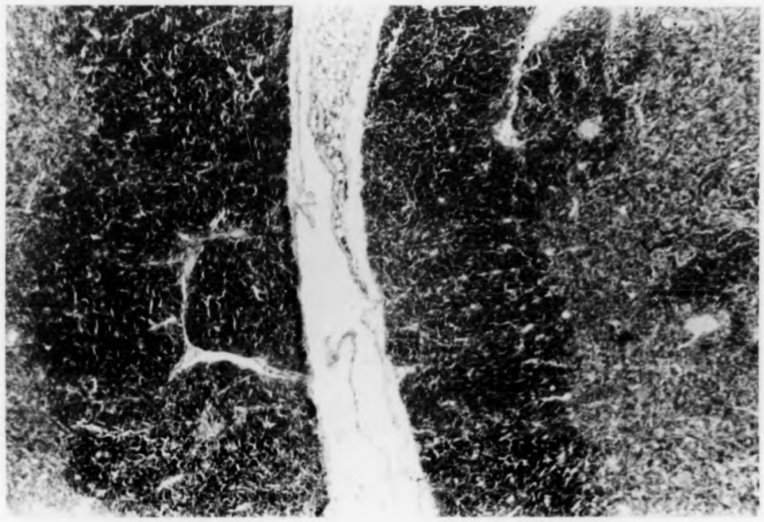
(Fig. 2.19c, Table 2.19 and Plates 2.23 to 2.26)

Thymus weight and thymus weight : body weight ratio showed a progressive decline over the first 17 days of infection. On day 17 the thymus weight : body weight ratio was 1.37 compared to 6.40 on day 0. By day 27 there was some recovery and on day 59, i.e. 22 days after the elimination of the infection, the thymus weight : body

Plate 2.23 Thymus of normal uninfected T.O. mouse. H. & E. x 70

Plates 2.24, 2.25, 2.26 Thymus of T.O. mice infected with P. b. yoelii, showing erosion of the thymic cortex.

Plate 2.24 Day 15 of infection. H. & E. x 70



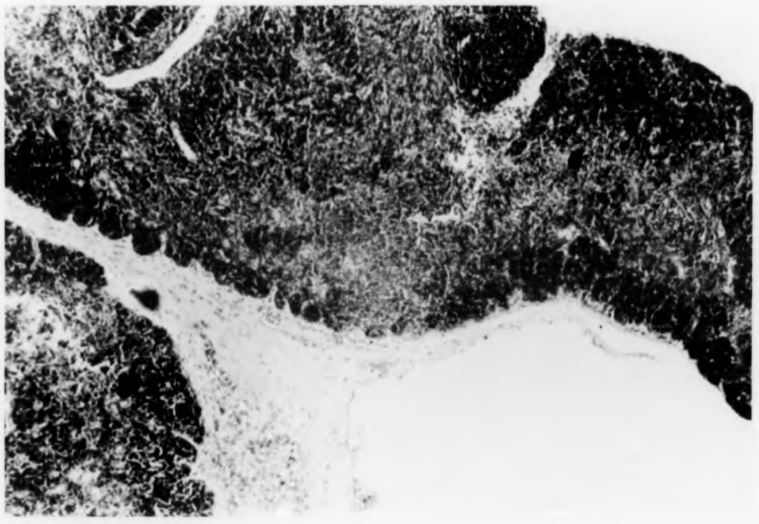
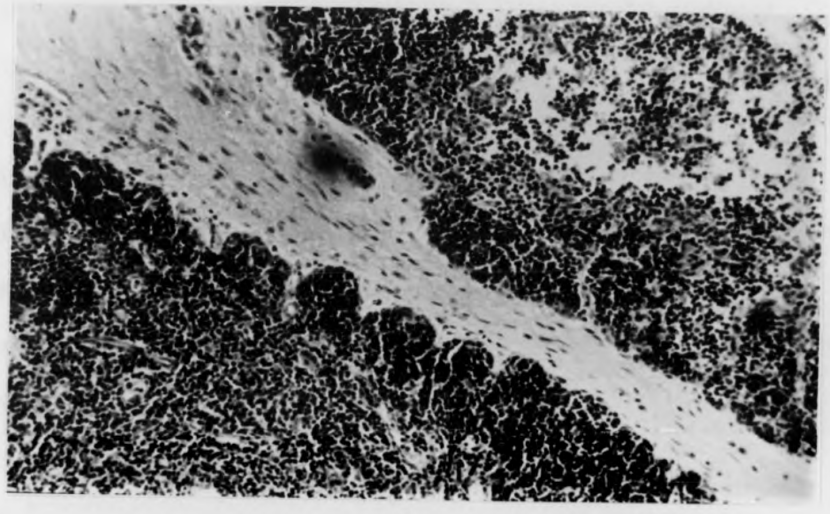
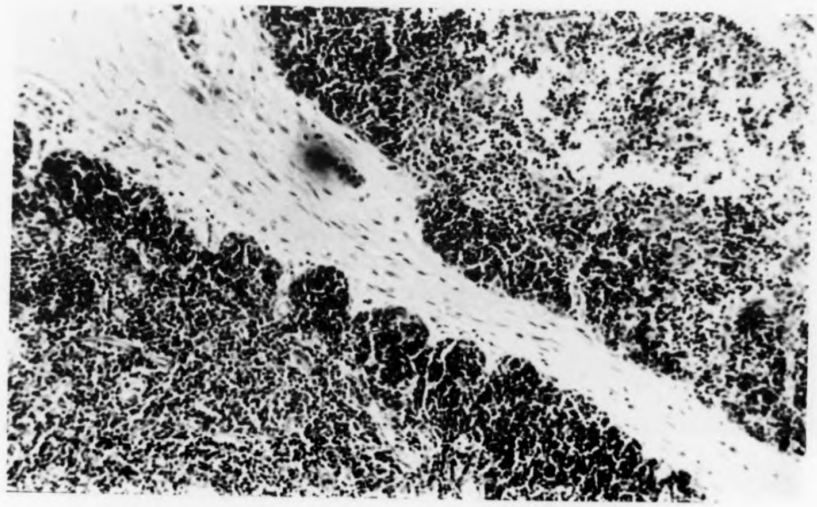
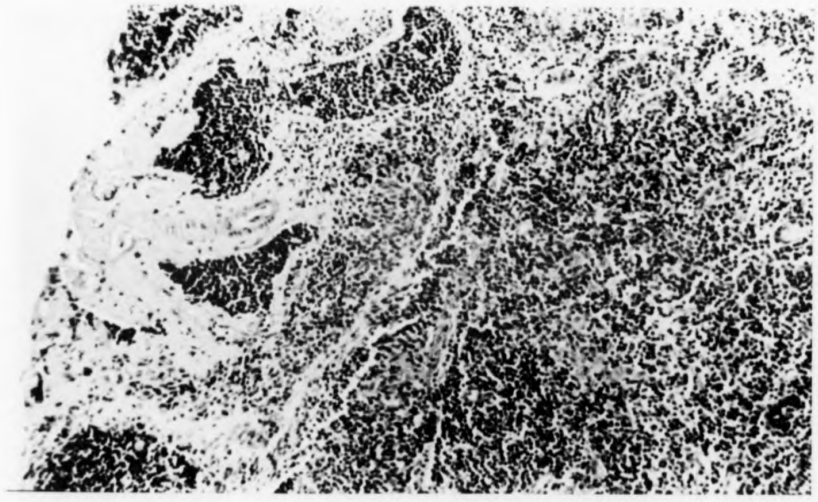


Plate 2.25 Day 15 of infection. H. & E. x 196

Plate 2.26 Day 19 of infection. H. & E. x 196







weight ratio had increased to 11.11, which was significantly higher than the value recorded on day 0, or in a group of age matched controls sacrificed at the same time.

Histologically, over the first 8 days of infection, the thymus appeared normal. By day 11 there was slight denudation of the thymic cortex. Between day 14 and day 19, pronounced destruction of the thymic cortex was observed. There was no evidence of regeneration at this stage. With the elimination of the parasitaemia the normal architecture of the thymus was rapidly restored.

D. Adrenals

(Figs. 2.19c and Table 2.19)

Adrenal weights and the adrenal weight : body weight ratio showed a substantial increase during the infection. On day 17 and 27 the adrenal weight : body weight ratios were more than twice the value recorded on day 0.

Histologically, at day 9, a slight expansion of the adrenal cortex was observed. This trend continued and by day 14 there was a marked increase in the size of the adrenal cortex. All three component zones of the adrenal cortex, the zonas glomerulosa, fasciculata and reticularis were expanded, and better defined than in uninfected controls. The vascular channels in the cortex were also clearly defined in the infected mice. A few lipid vacuoles were present. Some haemozoin pigment was seen. At day 27 the adrenal cortex was still well defined, but appeared less active than at day 17.

1.3 Fluorescent antibody levels

(Table 2.20)

Fluorescent antibody determinations were carried out on pooled serum samples obtained on day 0, 5 and 14 of infection. The results are summarised in Table 2.20. No IgG_1 was detected; low titres of IgG_2 were present on day 14. IgM was present at a titre of 16 on day 5 and 14.

1.4 Steroid levels

To determine whether P. b. yoelii infections caused any significant change in plasma corticosterone levels in T.O. mice, a separate experiment was carried out. A group of T.O. mice aged 12 weeks, were infected with 1×10^4 parasitized RBC. On day 14 of infection when the mean percentage parasitaemia was 17%, plasma samples were collected for plasma corticosterone determinations from the infected mice and from a group of uninfected controls, which had been placed under identical conditions as the infected group during the experiment. Body weights of these mice were recorded; they were then killed by cervical dislocation and organ weights of the adrenals, thymus, spleen and axillary lymph nodes were obtained. The tissues were processed for histology.

The results of this experiment are summarised in Table 2.21. The plasma corticosterone levels and organ weights of individual mice and mean values $\pm$ 1 S.E. of the groups are shown to indicate the limited amount of intra-group variation found in these experiments with T.O. mice. The responses of inbred CBA mice

were found to be even more uniform, and the sampling of a limited number of mice for organ weight determinations was probably justified.

The plasma corticosterone levels of the infected group were significantly increased ($p < 0.001$). Adrenal weight : body weight ratios in the infected group were 8.9 compared to 5.2 in the controls. Thymus weight : body weight ratios were significantly reduced in the infected group - 3.21, compared to 8.85 in the controls ($p < 0.05$).

2. THE RESPONSE OF T.O. MICE TO P.B.BERGHEI

2.1 Parasitaemias

(Fig. 2.20 and Table 2.22)

After the inoculation of 1×10^4 P. b. berghei parasitized RBC parasites were detected in the peripheral blood by day 2. Between day 2 and day 5 the parasitaemia showed a logarithmic increase, and by day 7 of infection 17.45% of the RBC were parasitized. The infection proved fatal in all mice between day 8 and day 10. The mean percentage parasitaemia recorded on day 8 was 22.64. The terminal parasitaemias ranged between 20% and 40%.

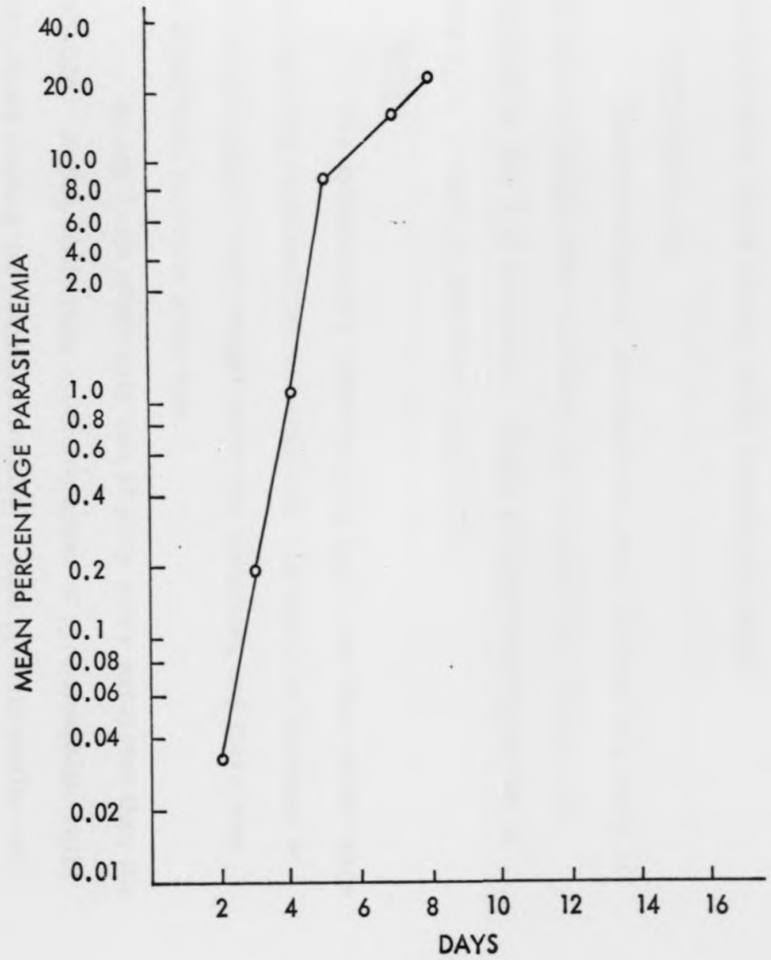
2.2 Haematology

(Table 2.23)

Total and Differential Nucleated Cell Counts

The resting (day 0) leukocyte levels in outbred T.O. mice

Fig. 2.20 Mean percentage parasitaemias in outbred T.O. mice infected with 1×10^4 P.b. berghei parasitized RBC.


MEAN PERCENTAGE PARASITAEMIA
40.0
20.0
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
DAYS

were found to be much higher than in CBA mice. On day 6 of infection the total nucleated cell count was lower than on day 0, but during the terminal stages of the infection it was raised. Polymorph and monocyte counts showed a substantial increase by day 8, but there appeared to be little change in the lymphocyte count.

2.3 Immunopathology

Histopathological investigations were limited to a study of the spleen, lymph nodes (axillary and mesenteric), thymus and adrenals on day 7 of infection. Organ weights were recorded on days 0, 3, 7 and 9 of infection (Table 2.24).

A. Spleen

The splenomegaly observed was much less than in the other host parasite combinations investigated. On day 3 an increase in the spleen weight : body weight ratio was observed, but there was no significant increase after this.

At day 7 the white pulp was slightly more extensive than the red pulp. In the white pulp extensive zones of pyroninophilic cells were found cuffing the central arteriole. This pyroninophilic cell population was similar to that seen previously in other infected spleens. Some germinal centre development was observed but these structures did not appear very active. The red pulp showed haematopoietic activity and contained many haemozoin containing macrophages. Some pyroninophilic cells including plasma cells were seen in the red pulp; many large megakaryocyte like cells were also observed.

B. Lymph Nodes

Axillary node weights increased during the infection. Histopathologically no changes were detected in either the axillary or mesenteric nodes.

C. Thymus

Thymus weight : body weight ratios declined sharply from 18.00 on day 0, to 4.70 on day 9. By day 7 of infection the thymus showed slight depletion of the cortex, but the extent of depletion was much less than observed by day 15 of a P. b. yoelii infection in the T.O. mouse, or in P. b. berghei infections in the CBA mouse. No changes were detected in the thymic medulla.

D. Adrenals

Adrenal weights showed a slight increase during the infection. The adrenal cortex in infected mice appeared better defined than in uninfected controls.

2.4 Fluorescent antibody levels

(Table 2.25)

Antiplasmodial antibodies were measured on pooled serum samples obtained on days 0, 3 and 7 of infection. IgM was detected at titres of 32 and 64 on days 3 and 7, respectively. No IgG_1 or IgG_2 was detected.

DISCUSSION

The experiments described in this chapter show that both P. b. yoelii and P. b. berghei infections in the mouse provide extremely valuable model systems for studying in vivo immune mechanisms in experimental malaria. An attempt will be made to discuss the most significant aspects of the interaction between these two parasites and their hosts.

1. P. b. yoelii in the CBA mouse, being a non-fatal infection, provides a particularly valuable model for analysing the mechanism of acquired immunity in malaria. This parasite showed a very characteristic and reproducible infection in the CBA mouse lasting 15-16 days, after which the animal was immune to re-infection. It had been claimed that the immunity developed in P. b. yoelii infections in the mouse was sterile, i.e. immunity was maintained in the absence of persisting antigen (Barker, 1971). The demonstration of parasites in the kidneys of CBA mice 4 weeks after the regression of the blood parasitaemia, at a time when parasites could not be detected in any other tissue examined, brings into question the claim of Barker. The precise mechanism by which the parasites persist in the immune host and the biological significance of such persistence are uncertain. It is possible that the kidney acts as an immunologically privileged site in which these organisms are sheltered from the "primed" immune system. The observation of Viens et al. (1973) who found that T. musculi also persisted in the kidneys of mice for up to one year after infection would be compatible with such an interpretation.

The persistence of these organisms could also be of importance in the maintenance of immunity by the recovered host. It is possible that the release of small amounts of antigen from this "reservoir" might be the stimulus by which the immunological defences of the host are maintained in a primed condition. The inability to break this immunity with anti-thymocyte serum (ATS) despite the susceptibility of the primary response to such treatment, suggests that the cells involved in maintaining immunity are non recirculating cells which escape the effects of ATS, or that there is constant production of such cells due to the effects of the persistent antigen on a precursor cell, as a result of which this population cannot effectively be depleted by ATS treatment.

The occurrence of a sterile immunity has been reported in other host parasite combinations as well, e.g. P. b. berghei in the rat (Corradetti, 1963) and P. cynomolgi bastianelli in the rhesus monkey (cited by Cohen, 1974). In view of the present findings it would seem opportune to re-examine the nature of the immunity developed in these situations.

2. The immune response to P. b. yoelii was shown to be thymus dependent. While intact mice controlled and cleared the infection effectively, infections were fulminant and fatal in all T cell deprived mice and in 70% of the ATS treated mice. The results of the T cell deprivation experiments are probably the clearest demonstration of complete T cell dependence in a malarial infection. The limited degree of thymic dependence demonstrated previously using

P. b. berghei infections in neonatally thymectomized rats (Brown, Allison and Taylor, 1968; Stecschulte, 1969) could in part be due to the incomplete effects of thymectomy in this species (Ogilvie and Jones, 1973),

The histological and serological studies carried out on normal and deprived mice infected with P. b. yoelii were strongly suggestive of a thymus dependent humoral antibody response being of key importance in the immune response to the parasite. While the normal CBA mouse showed a vigorous and sustained germinal centre response in the spleen, and produced high levels of IgG_1, IgG_2 and IgM antibodies, in the deprived mouse the germinal centre response was severely impaired and the production of IgG_1 type antibodies was almost completely abolished. The levels of IgG_2 and IgM were also lower than in normal mice, but their production was affected to a much lesser extent than IgG_1. Such a differential effect of T cell deprivation on Ig classes has been reported with other antigens and the degree of inhibition of antibody synthesis in T cell deprived mice is attributed,in part, to the nature of the antigen used (Miller and Mitchell, 1969).

A close relationship is now thought to exist between thymic influence, germinal centre formation and 7S antibody production. De Sousa et al. (1969) and Mitchell et al. (1972) showed that the congenitally athymic nude mouse lacked germinal centres thus indicating that the thymus played an important part in germinal centre development. More recently, Jacobson and Thorbecke (1973)

found that nude mice challenged with Brucella abortus produced low titres of 7S antibody and lacked germinal centres but when these mice were injected with thymus cells they showed a good 7S response and germinal centres developed in the spleen and lymph nodes. Germinal centres are also thought to be important in the development of immunological memory (Thorbecke, 1969) particularly of the IgG class (Jacobson and Thorbecke, 1968) and in the mouse the germinal centre response has been linked in particular to the production of IgG_1 (Asofsky, cited by Jacobson et al., 1974). The sensitivity of IgG_1 antibody production to thymic influence has previously been demonstrated by Taylor and Wortis (1968) and Torrigiani (1972) who found that IgG_1 levels were markedly depressed in thymectomized mice. Luzzati and Jacobson (1972), Bloemmen and Eyssen(1973), Pritchard et al. (1973) have reported similar findings in the congenitally athymic nude mouse.

Further evidence for the occurrence of a close relationship between T cell competence, germinal centre activity and the development of a protective response in P. b. yoelii infections was obtained by reconstituting T cell deprived mice with thymus grafts to different degrees of T cell competence; the degree of protective immunity and the germinal centre response elicited in these mice correlated closely with the extent of reconstitution achieved by the implantation of syngeneic thymus grafts. Precisely how T cells influence the formation of germinal centres is not clear, but these structures, which are mainly of bone marrow origin, have been shown

to contain a few T cells by Guttman and Weissmaan (1972). The possibility that a humoral factor (thymus hormone) might be involved has not been excluded.

3. Unlike P. b. yoelii, P. b. berghei induced a progressive infection which killed normal CBA mice within 25 days. Compared to P. b. yoelii infections it provoked a weak germinal centre response, and lower levels of antibody particularly of the IgG_1 class were produced. It was shown (in experiments described in the next chapter) that P. b. berghei induced less reactivity in the T cell pool than P. b. yoelii and the reduced germinal centre activity and antibody production observed were probably due to this diminished reactivity in the T cell pool. The methods by which the parasite might have influenced the T cell pool are discussed together with the relevant experiments in Chapter 3.

However, the apparently inadequate humoral response to P. b. berghei might not be due solely to the production of less antiplasmodial antibody - it might also be due to a qualitative defect in the type of antibody produced. Steward and Voller (1973) reported that the ability of Simpson mice infected with P. b. yoelii to produce antibody to Human Serum Transferrin (HSF) was unimpaired in terms of the quantity of antibody produced but the antibody produced was of lower affinity than in uninfected controls. Further, these workers suggested that individuals capable of making high affinity antibodies may, when infected with malaria, produce low affinity antibodies to the malarial antigens as well. The affinity of antibodies are known

to be important in immune elimination of antigen, low affinity antibodies being less efficient than high affinity antibodies (Alpers et al., 1972). It is conceivable that P. b. berghei infections result not only in a quantitative defect in antibody production, but also in the production of antibodies of even lower affinity than in P. b. yoelii which are consequently less effective. It is thought that high affinity antibodies are more T cell dependent than low affinity antibodies (see Mitchell, 1974) and the reduced reactivity in the T cell pool might not favour their production in P. b. berghei infections.

4. In addition to the reactive changes seen in the germinal centres, the spleens of infected mice also showed marked changes in the periarteriolar regions of the spleen. These areas were designated 'thymus dependent' (Parrott et al., 1966) but recently Mitchell (1973) has suggested that they may not be entirely T cell dependent.

P. b. yoelii and P. b. berghei infections in both CBA and outbred T.O. mice resulted in the production of pyroninophilic cell infiltrates in this area. This response paralleled the pattern of infection. In normal CBA mice infected with P. b. yoelii it was maximal at the peak of infection after which it gradually receded as the parasitaemia regressed. In P. b. berghei infections it progressed with the parasitaemia and during the terminal stages of the infection massive pyroninophilic cell infiltrates were seen around the central arteriole. In P. b. yoelii infected deprived mice the response developed more slowly and was less extensive initially, but during

the terminal stages of infection considerable pyroninophilia was observed. Therefore, this response by itself showed no obvious correlation with the development of immunity.

The main cell type which contributed to this infiltrate was a large indeterminate, pyroninophilic cell. Some immature and mature plasma cells were also seen. It is possible that some of these morphologically indistinguishable cell types formed a functionally heterogeneous population - their functional activity varying during an infection, and in the different host parasite combinations studied. Moran et al. (1973) observed a similar pyroninophilic response in Balb/C mice infected with P. b. yoelii and found that from day 6 onwards, many of the cells contained IgG. They failed to detect any IgM containing cells in this area, and reported that such cells were confined to the red pulp. In the course of the present investigation it was shown that P. b. yoelii triggered off a sustained burst of T cell activity during the infection. P. b. berghei, on the other hand, induced much less activity in the T cell pool. It is likely therefore, that this is the site of both T and non-T cell responses, with the proportion of T to non-T cells varying in different situations.

Other factors might also have contributed to the accumulation of these cells in large numbers in the periarteriolar regions, and for the paucity of small lymphocytes in these areas during an infection. Mitchell (1973) has demonstrated that the exit of cells from these regions occurs through a series of fine channels which

extend across the marginal zone; she has also suggested that pseudolymphatics located in the periarteriolar regions might serve as an outlet. These channels have a definite reticulin framework, and disruption of this framework during the infection, could restrict the normal pattern of lymphocyte movement in the spleen, and give rise to the histopathological picture seen.

5. Both P. b. yoelii and P. b. berghei infections were shown to have significant effects on the thymus. In general, the extent to which the thymus was affected depended on the duration and severity of the infection. In mild, self-limiting infections (P. b. yoelii in the CBA mouse), although decreased thymus weights were recorded, histologically no changes were observed. In acute infections such as P. b. yoelii in the T.O. mouse and P. b. berghei in the CBA mouse, the effects were more pronounced, and consisted mainly of depletion of the thymic cortex. These infections were also shown to cause significantly increased steroid levels and adrenal activity. In P. b. yoelii infections in the CBA mouse the rise in plasma corticosterone was temporary, and hence appeared to have relatively little effect on the thymus. In acute infections the increased steroid levels were probably maintained over longer periods of time due to the severity and time course of these infections, and was likely to have been the main factor responsible for the changes seen in the thymus. The lympholytic effects of steroids are now well established; cells of the thymic cortex have been shown to be most sensitive to the action of steroids (Warner, 1964; Blomgren and Anderson, 1969;

Cohen and Claman, 1971). These cells have been designated cortisone sensitive in contrast to the cortisone resistant medullary lymphocytes.

The deleterious effects of these infections on the thymus are probably an important factor in the non-specific immunodepression observed during malarial infections, the degree of immunodepression usually correlating well with the severity of the infection. As the cells most affected appear to be the thymic cortical lymphocytes - the source of T_1 cells (Cantor and Asofsky, 1972) - it seems likely that responses either directly or indirectly dependent on a T_1 type cell (which is a short lived non-recirculating cell) are most likely to be depressed due to the thymic cortical depletion found in acute malarial infections.

6. Comparison of the immunological responses of the CBA mouse with the outbred T.O. mouse showed that the T.O. mouse was a relatively poor responder; both P. b. yoelii and P. b. berghei infections were more severe in this strain of mouse. P. b. yoelii infections which were mild and non fatal in the CBA mouse, resulted in a comparatively severe infection which killed 40% of T.O. mice. P. b. berghei infections were fatal in both species but while the mean survival time in the CBA mouse was 22 days, in the T.O. mouse it was 7.5 days. Different strains of mice appear to show different degrees of susceptibility to malarial infections. Parkes, C_3H and Sha-Sha strains of mice show varying levels of susceptibility to P. b. yoelii (Jayawardena, unpublished results). Strain-specific

susceptibility has also been reported in P. berghei infections (W.H.O., 1968).

Thus, the immune response to malaria appears to vary considerably in different hosts; as the ability of a host to respond is largely a function of its immunocompetent cells (including T cells) there must be a mechanism by which such variation in host immunocompetence is controlled. A growing body of evidence suggests that genetic factors determine such variability in immune responsiveness and histocompatibility linked immune response genes (Ir genes) are thought to represent differences in the ability of high and low responders to recognize immunogens.

Evidence for the importance of genetic factors in immunity to malaria is also implied in certain observations on human malaria. Resistance to P. vivax has been shown to be significantly higher in American negroes than in Caucasians, independent of previous exposure and red cell abnormalities (Young et al., 1955; Contacos et al., 1970). More recently, evidence has been obtained for a possible relationship between resistance to malaria and the HL-A system (the major histocompatibility system of man) (Piazza et al., 1973; Ceppellini et al., 1973). These workers studied HL-A variation in a group of Sardinian villages some of which had been malarious until recently, and others which had always been free of malaria, and demonstrated a relationship between HL-A variation and the distribution of malaria.

There has been increasing evidence for direct relationships

between predisposition to disease (both infectious and malignant) and histocompatibility linked Ir genes. For instance, a linkage has been established between the susceptibility of mice to leukaemia (Lilly, 1973) and the H-2 system (the major histocompatibility system in the mouse). It seems highly likely that a similar relationship might obtain in malaria and it would seem extremely important to establish the precise nature of such a relationship in both the experimental and human situations.

Chapter 2 Tables

Table 2.1 Mean percentage parasitaemias ± 1 S.E. in normal, sham-deprived (sham), and T-cell deprived (deprived) mice inoculated with 1×10^4 P. b. yoelii parasitized RBC.

Days	3	4	6	8	10	12	13	15	16	17
Normal	0.06 ±0.01	0.53 ±0.05	1.16 ±0.09	1.77 ±0.11	5.15 ±0.57	4.63 ±1.23	2.85 ±1.19	0.07 ±0.06	-	-
Sham	0.05 ±0.01	0.45 ±0.09	1.45 ±0.15	2.02 ±0.20	5.03 ±0.75	3.46 ±0.77	4.01 ±1.55	1.96 ±0.23	0.05 ±0.02	-
Deprived	0.33 ±0.04	0.63 ±0.07	1.29 ±0.11	1.85 ±0.14	1.82 ±0.13	3.96 ±0.13	3.99 ±0.19	5.02 ±0.38	9.94 ±0.73	-

Days	18	20	22	24	25	27	29	30	33	34
Normal	-	-	-	-	-	-	-	-	-	-
Sham	-	-	-	-	-	-	-	-	-	-
Deprived	18.57 ±2.09	15.45 ±1.82	28.07 ±5.45	36.04 ±1.79	40.00 ±2.26	39.81 ±6.69	40.34 ±2.52	56.35 ±3.75	69.89 ±7.26	79.66 ±6.33

Table 2.2 Mean percentage parasitaemias* in rabbit anti-mouse thymocyte serum (ATS) treated normal and T-cell deprived mice, and in normal rabbit serum (NRS) treated normal and deprived mice. Inoculum 1×10^4 P. b. yoelii parasitized RBC.

Days	3	5	8	11	12	13	14	16	18	21	25	27	29	31	33	35	37	39	41	46	52
Norm. Untr.	0.20	0.20	1.00	8.20	10.90	11.80	10.70	6.80	0.07	–											
Norm. NRS	–	–	–	0.04	0.60	1.42	2.30	4.50	6.40	8.00	3.12	0.30	–								
Norm. ATS	–	–	–	0.38	2.20	8.70	8.50	10.80	12.40	29.60	38.50	41.40	43.20	46.00	38.50	55.70	62.20	55.50	68.00	40.00	–
Depr. Untr.	0.01	1.59	1.90	6.10	10.05	16.90	17.00	36.80	42.20	45.10	47.00	47.00	65.40	54.30	53.60						
Depr. NRS	–	–	0.02	0.60	2.80	ND	9.60	15.00	32.35	31.00	48.00	40.70	47.00	ND	ND						
Depr. ATS	–	–	–	2.40	4.80	5.00	7.70	17.80	15.90	45.70	42.10	41.00	43.50	47.00	46.70	59.50	ND	48.30			

* standard errors were not calculated.

Norm. = normal; Untr. = untreated; Depr. = deprived.

Table 2.3 Mean packed cell volumes (PCV) in normal, sham and T-cell deprived mice infected with P. b. yoelii

days	0	4	6	8	10	13	15	16	18	20	22	24	27	29	30	33	34
N	47.10	46.94	41.00	38.25	32.00	31.48	35.19	34.03	35.64	43.17	39.00	44.59	42.50	39.85	39.97	41.03	41.86
S	44.50	36.73	38.22	41.67	32.22	34.62	32.44	31.81	36.58	41.76	ND	38.89	44.44	ND	ND	ND	ND
D	45.50	40.00	40.81	41.00	35.30	34.78	30.77	ND	22.41	21.74	16.86	16.91	ND	12.50	15.69	8.96	10.29

Table 2.4 Mean percentage reticulocyte levels in normal, sham and T-cell deprived mice infected with P. b. yoelii

days	0	3	4	6	8	10	13	15	16	18	20	22	24	25	27	29	30	33	35
N	0.17	0.18	0.16	1.22	4.07	10.97	23.70	53.60	48.60	24.80	24.00	12.30	10.80	9.80	6.40	5.20	5.00	6.00	2.90
S	0.30	0.37	0.44	2.63	3.60	11.85	22.60	43.63	54.12	22.45	13.46	10.83	5.87	4.50	4.00	3.62	3.70	3.70	3.10
D	0.40	0.52	0.48	1.69	4.08	5.03	14.25	12.39	11.18	20.50	30.70	42.66	41.27	33.03	ND	ND	73.86	76.00	-

(ND = No Determination)

Table 2.5 Absolute numbers of nucleated cells/mm^3 ± 1 S.E. in the peripheral blood of normal, sham and T-cell deprived mice infected with P. b. yoelii

Table 2.5a Total nucleated cell count/mm^3

Day	0	3	4	6	8	10	13	16	18	20	22	25	27	29	33	35
Normal	5,400 ±476	7,500 ±405	18,500 ±1,000	52,700 ±5,294	40,825 ±5,175	36,033 ±1,116	55,200 ±4,424	37,000 ±5,150	26,550 ±3,420	19,416 ±3,873	16,656 ±536.4	20,150 ±2,627	15,450 ±1,050	16,700 ±3,393	16,100 ±600	15,000 ±925
Sham	6,600 ±385	9,750 ±325	13,925 ±325	29,750 ±1,679	39,566 ±2,627	52,133 ±3,678	43,366 ±4,050	42,250 ±2,422	21,950 ±5,588	17,100 ±2,250	23,400 ±2,814	24,250 ±6,050	16,100 ±600	15,250 ±4,693	22,100 ±2,300	15,600 ±2,100
Deprived	5,400 ±707	14,250 ±475	15,800 ±500	21,150 ±803	27,700 ±2,696	33,253 ±4,088	30,750 ±5,910	16,800 ±2,478	17,433 ±721	13,250 ±1,601	16,033 ±1,134	22,250 ±2,050	28,750 ±5,943	32,400 ±400	65,600 ±3,317	–

Table 2.5b Neutrophils/mm^3

Day	0	3	4	6	8	10	13	16	18	20	22	25	27	29	33	35
Normal	2,144 ± 243	5,329 ±417	8,600 ±429	18,112 ±2,108	16,348 ±1,020	15,080 ±945	20,357 ±2,727	18,525 ±2,039	10,448 ±1,060	8,359 ±695	8,631 ±496.37	7,409 ±800	6,756 ±695	7,974 ±616	8,290 ±215	6,801 ±1,680
Sham	3,100 ±95	6,880 ±312	6,581 ±646	13,362 ±2,369	13,162 ±1,226	22,479 ±1,508	13,898 ±2,553	18,581 ±2,280	9,462 ±299	7,703 ±939	10,530 ±407	8,770 ±285	7,840 ±334	8,443 ±643	ND	8,323 ±1,058
Deprived	3,205 ±346	8,575 ±448	7,877 ±726	8,199 ±272	18,860 ±341	20,105 ±2,662	12,743 ±1,296	8,910 ±279	9,299 ±671	8,241 ±634	8,956 ±720	11,894 ± 243	18,195 ± 983	18,493 ±1,965	41,977 ±3,340	–

Table 2.5c Lymphocytes/mm^3

Day	0	3	4	6	8	10	13	16	18	20	22	25	27	29	33	35
Normal	2,852 ±224	1,042 ±354	2,980 ±233	9,454 ±2,529	6,329 ±367	5,526 ±425	9,450 ±1,860	11,388 ±1,163	9,528 ±1,420	5,156 ±793	6,202 ±277	9,450 ±1,585	7,083 ±1,068	6,364 ±876	7,106 ±398	6,862 ±1,680
Sham	2,900 ±166	1,507 ±241	2,887 ±733	5,503 ±1,763	9,199 ±1,248	13,225 ±1,525	8,600 ±2,785	10,304 ±1,398	9,023 ±501	4,794 ±336	9,594 ±772	7,941 ±314	6,287 ±795	4,748 ±1,007	ND	7,015 ±2,566
Deprived	1,204 ±46	1,987 ±521	2,840 ±347	3,142 ±1,808	3,941 ±1,071	6,098 ±885	3,375 ±723	3,134 ±545	4,503 ±1,089	2,997 ±319	2,698 ±456	7,355 ±1,410	5,123 ±677	4,966 ±1,001	14,740 ±1,252	–

Table 2.5d Monocytes/mm^3

Day	0	3	4	6	8	10	13	16	18	20	22	25	27	29	33	35
Normal	380 ±71	1,125 ±106	5,960 ±484	24,126 ±368	18,119 ±1,340	14,238 ±2,325	29,206 ±4,635	5,945 ±1,302	6,277 ±1,224	5,801 ±1,593	1,530 ±331	3,284 ±1,221	1,603 ±559	2,356	1,216 ±441	1,060 ±330
Sham	489 ±108	1,330 ±302	4,153 ±917	10,126 ±2,761	16,705 ±269	14,852 ±2,400	18,978 ±6,324	12,941 ±2,517	3,457 ±106	3,281 ±806	3,346 ±53.8	5,451 ±289	1,928 ±819	3,040 ±473	ND	325
Deprived	895 ±115	3,461 ±319	3,374 ±834	7,095 ±2,622	11,150 ±1,023	8,711 ±2,134	14,771 ±848	4,720 ±420	3,524 ±745	1,876 ±446	4,253 ±827	2,997 ±150	5,637 ±1,017	8,122 ±2,154	8,646 ±2,275	–

Table 2.6 Mean spleen weight and spleen weight/body weight ratio in normal, sham and T-cell deprived mice infected with P. b. yoelii

Spleen weights (gms)

days	0	7	14	21	28	35
Normal	0.0941	0.3283	1.1850	0.3522	0.2048	0.1800
Sham	0.0957	0.3242	0.6138	0.1751	0.1146	0.2600
Deprived	0.0890	0.1700	0.4227	0.4548	0.4113	0.3650
Spleen weight/body weight x 10^3						
Normal	2.80	5.80	14.83	17.70	18.20	20.00
Sham	2.88	10.99	18.60	5.80	3.70	2.40
Deprived	2.80	9.30	35.40	10.50	5.60	5.80

Table 2.7 Mean axillary lymph node weight and lymph node weight/body weight ratio in normal, sham and T-cell deprived mice infected with P. b. yoelii

Axillary lymph node weights (gms)

days	0	7	14	21	28	35
Normal	0.0030	0.0183	0.0152	0.0145	0.0050	0.0058
Sham	0.0026	0.0090	0.0162	0.0088	0.0048	0.0057
Deprived	0.0019	0.0024	0.0028	0.0028	0.0018	0.0009
Axillary lymph node weight/body weight x 10^4						
Normal	0.90	5.19	4.56	4.44	1.37	1.44
Sham	0.80	3.02	4.99	2.63	1.58	1.40
Deprived	0.60	0.64	0.94	1.10	0.82	0.43

Table 2.8 Fluorescent antibody levels (expressed as the reciprocal of the last dilution showing a + fluorescence in normal, sham and T-cell deprived mice infected with P. b. yoelii

Day	Total Ig			IgG_1			IgG_2			IgM			IgA		
	Norm.	Sham	Dep.	Norm.	Sham	Dep.	Norm.	Sham	Dep.	Norm.	Sham	Dep.	Norm.	Sham	Dep.
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	32	32	-	-	-	-	32	16	-	256	256	32	-	-	-
14	1024	1024	16	512	128	-	2048	1024	8	128	256	32	16	8	-
21	1024	1024	8	128	256	8	512	512	64	256	256	32	16	16	-
28	1024	1024	16	256	256	-	1024	1024	128	256	256	128	16	8	-
35	1024	1024	32	256	512	-	512	1024	128	128	128	64	16	16	-

Table 2.9 Serum immunoglobulin levels (expressed as a percentage of a standard serum) in normal and T-cell deprived mice infected with P. b. yoelii

Day	IgG_1		IgG_2		IgM	
	Norm.	Dep.	Norm.	Dep.	Norm.	Dep.
0	15.0	23.0	62.5	65.5	55.0	25.5
7	23.0	15.0	75.0	104.0	142.0	55.0
14	55.0	25.0	195.0	125.0	92.5	92.5
21	52.5	21.0	180.0	90.0	120.0	62.5
28	35.0	25.0	150.0	150.0	128.0	72.5
35	27.0	28.5	141.0	95.5	55.0	25.5

Table 2.10 Plasma corticosterone levels, thymus and adrenal weights in normal CBA mice infected with P. b. yoelii

2.10a Concentration of plasma corticosterone (µgs/100 ml)

day	0	6	9	14	16	20	29
Control	9.5	12.7	13.2	7.7	9.0	11	6.97
Infected	9.5	10.0	16.9	14.5	19.8	16	7.8

2.10b Thymus weight; thymus weight/body weight ratio

Thymus weight (gms)

day	0	6	9	14	16	20	29
Control	0.0367	0.0374	0.0317	0.0339	0.0352	0.0391	0.0315
Infected	0.0367	0.0482	0.0222	0.0325	0.0305	0.0314	0.0357
Thymus weight/body weight x 10^4							
Control	17.5	17.25	13.71	15.9	16.00	17.77	13.06
Infected	17.5	22.75	11.09	14.67	14.15	13.63	15.26

2.10c Adrenal weight; adrenal weight/body weight ratio

Adrenal weight (gms)

day	0	6	9	14	16	20	29
Control	0.0026	0.00265	0.0029	0.00262	ND	0.00230	0.0022
Infected	0.0026	0.00322	0.00315	0.00330	0.00370	0.00307	0.0025
Adrenal weight/body weight x 10^5							
Control	12.0	12.0	12.5	12.35	ND	10.4	9.2
Infected	12.0	15.1	15.0	14.8	15.4	13.1	10.7

Table 2.11 Mean percentage parasitaemia ±1 S.E. in P. b. yoelii infected T cell deprived mice reconstituted with thymus grafts 30 days before infection (group A), 15 days before infection (group B), or on the day of infection (group C).

Day	4	5	6	9	10	11	12	13	14	17	18	19	20
Reconstituted 30 days before infection (Gp. A)	0.07 ±0.01	0.50 ±0.40	1.37 ±0.43	2.14 ±0.45	3.40 ±0.84	6.52 ±2.53	5.67 ±1.97	2.54 ±0.90	18.17 ±4.48	3.53 ±1.21	1.97 ±0.85	1.35 ±1.13	0.49 ±0.48
Reconstituted 15 days before infection (Gp. B)	0.03 ±0.01	0.53 ±0.26	1.09 ±0.49	1.01 ±0.34	1.83 ±0.41	2.32 ±0.57	1.82 ±0.51	4.50 ±0.83	6.39 ±1.59	4.58 ±1.15	4.28 ±1.68	10.16 ±5.71	9.42 ±6.50
Reconstituted on day of infection (Gp. C)	0.05 ±0.02	ND	0.94 ±0.36	2.59 ±0.60	3.60 ±0.62	3.74 ±0.82	3.50 ±0.63	7.66 ±2.01	14.42 ±2.61	8.23 ±0.88	8.34 ±1.47	23.68 ±4.54	16.37 ±5.01

Day	21	23	25	26	29
Reconstituted 30 days before infection (Gp. A)	0.20 ±0.20	-			
Reconstituted 15 days before infection (Gp. B)	4.58 ±3.80	ND	0.71 ±0.21	-	
Reconstituted on day of infection (Gp. C)	11.42 ±4.70	12.97 ±4.8	ND	4.30 ±1.32	-

Table 2.12 Mean percentage parasitaemias ± 1 S.E. in normal CBA mice infected with 1×10^4 *P. b. berghei* parasitized RBC

Day	2	3	4	5	7	8
Percentage parasitaemia ± 1 S.E.	0.02 ±0.004	0.14 ±0.005	0.41 ±0.06	0.99 ±0.26	1.88 ±0.53	2.67 ±0.56

Day	9	10	12	15	16	18
Percentage parasitaemia ± 1 S.E.	3.10 ±0.53	4.75 ±1.18	7.69 ±1.88	15.79 ±2.43	14.32 ±2.70	27.60 ±4.08

Day	22	23
Percentage parasitaemia ± 1 S.E.	22.04 ±0.42	46.19 ±14.17

Table 2.13 Mean packed cell volume (PCV) ± 1 S.E. in normal CBA mice infected with P. b. berghei

Day	0	2	6	13	16	22
Packed cell volume ± 1 S.E.	48.16 ±1.93	43.75 ±2.27	39.09 ±3.41	24.58 ±1.78	18.16 ±3.28	11.08 ±0.32

Table 2.14 Absolute numbers of nucleated cells per mm^3 ± 1 S.E. (total nucleated cell count, neutrophils, lymphocytes and monocytes) in normal CBA mice infected with P. b. berghei

Day	0	1	6	8	13	15	20	22
Total nucleated cell count	9,350 ±1,950	14,133 ±1,215	11,480 ±1,105	15,340 ±2,493	25,125 ±3,288	17,375 ±3,171	30,500 ±4,050	32,250 ±1,850
Neutrophils	5,150 ±640	8,261 ±902	4,518 ±747	9,398 ±1,530	13,154 ±1,804	10,052 ±2,700	17,075 ±1,150	17,958 ±308
Lymphocytes	3,800 ±462	5,470 ±688	5,403 ±826	3,999 ±965	9,045 ±2,417	6,390 ±990	9,134 ±2,763	11,672 ± 92
Monocytes	400 ±62	412 ±78	288 ±63	657 ±137	2,872 ±483	928 ±146	2,480 ±680	2,632 ±1,654

Table 2.15 Organ weights (spleen, axillary lymph nodes, thymus and adrenals), and organ weight/body weight ratios in normal CBA mice infected with P. b. berghei

Day	0	5	9	15	18
Spleen weight	0.1020	0.1553	0.4318	0.6596	0.6958
Spleen weight/ body weight x 10^3	3.00	4.58	13.70	23.50	24.40
Axillary lymph node weight	0.0032	0.0167	0.0141	0.0159	0.0129
Lymph node wt/ body weight x 10^4	0.96	4.19	4.50	6.24	4.52
Thymus weight	0.0272	0.0194	0.0070	0.0092	0.0045
Thymus weight/ body weight x 10^4	8.00	5.67	3.40	3.17	1.58
Adrenal weight	0.0025	0.0027	0.0041	0.0033	0.0047
Adrenal weight/ body weight x 10^5	7.10	8.40	13.00	12.90	16.40

Table 2.16 Fluorescent antibody levels (expressed as the reciprocal of the last dilution showing a + fluorescence) in normal CBA mice infected with P. b. berghei

Day	Total Ig	IgG_1	IgG_2	IgM	IgA
0	-	-	-	-	-
5	-	-	-	32	-
9	32	16	32	128	-
15	32	32	64	256	-
18	64	128	128	64	-
24	64	128	256	64	-

Table 2.17 Serum immunoglobulin levels (expressed as a percentage of a standard serum) in normal CBA mice infected with P. b. berghei

Day	IgG_1	IgG_2	IgM
0	9.00	45.00	25.50
5	19.00	55.00	77.00
9	20.00	88.50	70.00
15	31.00	140.00	77.00
18	54.00	152.50	90.00
24	70.00	156.50	55.00

Table 2.18 Mean percentage parasitaemias ± 1 S.E. in outbred T.O. mice infected with P. b. yoelii

Day	3	5	8	9	11	13
Mean percentage parasitaemia	0.16 ±0.04	3.21 ±1.43	4.23 ±0.59	16.40 ±1.57	16.47 ±1.57	15.28 ±2.18

Day	16	19	21	24	26	27
Mean percentage parasitaemia	34.92 ±5.97	56.30 ±7.87	40.72 ±11.28	2.01 ±0.57	0.02	-

Table 2.19 Organ weights, and organ weight/body weight ratios in outbred T.O. mice infected with P. b. yoelii

Day	0	5	10	17	27	59
Spleen weight	0.2506	0.7730	1.846	1.1894	0.9888	0.5181
Spleen weight/ body weight x 10^3	5.80	18.81	42.46	36.69	29.39	11.69
Axillary lymph node weight	0.0166	0.0187	0.0150	0.0144	0.0136	0.0150
Axillary LN wt/ body weight x 10^4	3.83	4.49	3.51	4.41	3.88	3.36
Thymus weight	0.0278	0.0227	0.0209	0.0044	0.0106	0.049
Thymus weight/ body weight x 10^4	6.40	5.44	4.80	1.37	3.06	11.11
Adrenal weight	0.0017	0.0027	0.0016	0.0040	0.0041	0.00357
Adrenal weight/ body weight x 10^5	4.00	6.50	3.80	9.10	9.50	7.90

Table 2.20 Fluorescent antibody levels in T.O. mice infected with P. b. yoelii

Day	Total Ig	IgG_1	IgG_2	IgM	IgA
5	-	-	-	16	-
14	16	-	16	16	-

Table 2.21 Plasmacorticosterone levels, organ weights and organ/body weight ratios in uninfected and P. b. yoelii infected (day 15) T.O. mice

Uninfected controls

	Plasma corticosterone levels in µgms/100 ml	Adrenal weight	Adrenal wt/ body wt. x 10^5	Thymus weight	Thymus wt/ body wt. x 10^4	Spleen wt/ body wt. x 10^3	Axillary LN wt/ body wt. x 10^4
1	8.80	0.0021	4.9	0.0401	9.43	5.80	3.20
2	13.20	0.0016	4.0	0.0304	8.80	4.35	3.30
3	9.50	0.0025	6.8	0.0304	8.32	3.87	2.60
4	12.50	0.0019	5.0	0.0360	8.87	5.00	2.90
Mean ± 1 S.E.	11.00 ±1.70	0.0020 ±0.00026	5.2 ±0.825	0.0352 ±0.00280	8.85 ±0.321	4.67 ±0.580	3.03 ±0.218

P. b. yoelii infected

1	20.40	0.0031	7.8	0.0156	3.94	43.18	3.18
2	26.20	0.0035	10.9	0.0109	3.40	41.45	3.34
3	11.40	0.0028	9.1	0.0058	1.90	42.50	3.10
4	26.40	0.0030	7.8	0.0137	3.60	38.85	4.89
5	24.30	0.0031	8.8	0.108	3.10	40.40	3.50
Mean ± 1 S.E.	21.74 ±3.519	0.0031 ±0.0001	8.9 ±0.7337	0.0115 ±0.0021	3.21 ±0.450	41.49 ±0.950	3.62 ±0.33

Table 2.22 Mean percentage parasitaemias ± 1 S.E. in outbred T.O. mice infected with P. b. berghei

Day	2	3	4	5	7	8
Mean percentage parasitaemia	0.033 ±0.01	0.206 ±0.035	1.19 ±0.097	9.10 ±1.21	17.45 ±2.71	25.43 ±9.53

Table 2.23 Absolute numbers of nucleated cells per mm^3 ± 1 S.E. in T.O. mice infected with P. b. berghei

Day	0	6	8
Total nucleated cell count	14,440 ±2,525	13,266 ±2,143	19,500 ±5,892
Neutrophils	5,833 ±1,251	3,715 ± 231	9,441 ±4,173
Lymphocytes	8,496 ±984	10,170 ±2,949	9,152 ±1,120
Monocytes	110 ± 48	645 ±129	829 ±108

Table 2.24 Organ weights, and organ weight/body weight ratios in T.O. mice infected with P. b. berghei

Day	0	3	7	9
Spleen weight	0.1905	0.2523	0.3201	0.2524
Spleen weight/ body wt. $\times 10^3$	6.35	8.42	11.63	9.33
Axillary lymph node weight	0.0080	0.00835	0.0095	0.0107
Axillary LN wt/ body wt. $\times 10^4$	2.66	2.76	3.45	4.10
Thymus weight	0.0540	0.0525	0.0206	0.0124
Thymus weight/ body wt. $\times 10^4$	18.00	17.39	7.50	4.70
Adrenal weight	0.0036	0.00385	0.00372	0.0040
Adrenal weight/ body wt. $\times 10^5$	12.00	12.7	13.5	13.8

Table 2.25 Fluorescent antibody levels in T.O. mice infected with P. b. berghei

Day	Total Ig	IgG_1	IgG_2	IgM	IgA
3	-	-	-	32	-
7	8	-	-	64	-

CHAPTER 3

THE MITOTIC RESPONSE OF THYMUS DERIVED CELLS

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INTRODUCTION

The ability of the CBA mouse to mount an effective immunological response against the murine malarial parasite P.b. yoelii was shown to be thymus dependent. Intact CBA mice were able to control the infection effectively, but "deprived" mice lacked the ability to limit the infection. It was also established that unlike P. b. yoelii, P. b. berghei infections always proved fatal in intact CBA mice within 20 to 25 days. The experiments reported in this section were carried out to define further the T cell response in non-fatal P. b. yoelii infections, and to compare it with the response in fatal P. b. berghei infections.

Use was made in this study of CBA/Lac → CBA/H.T6T6 mouse radiation chimaeras, bearing a chromosomally distinguishable population of T cells. Using similar analytical methods, Davies et al. (1966, 1969a & b, 1970) defined the responses of reconstituted CBA mice to antigens such as sheep red blood cells, Oxazolone, Salmonella flagellar antigen and Pneumococcal polysaccharide. More recently Weston et al. (1972) used the same approach to examine the immune response to allogenic skin and the EL4 lymphoma.

MATERIALS AND METHODS

1. A NOTE ON MOUSE RADIATION CHIMAERAS

Syngeneic mouse radiation chimaeras lacking a thymus of their own but having a thymus graft implanted under the kidney

capsule provide useful vehicles for studying the actions and interactions of thymus processed (T) and non thymus processed (B) lymphocyte populations (Davies, 1969c).

When a CBA/Lac (hereafter called Lac) mouse is thymectomized at 6 to 8 weeks of age and subsequently lethally irradiated and reconstituted with femoral bone marrow, the mouse is effectively depleted of its T cell population. If a thymus graft, consisting of a single thymus lobe from a CBA neonate, is implanted under the kidney capsule of such a "deprived" mouse it can be restored to a near normal level of immunocompetence (Leuchars et al., 1965). A graft consisting of two lobes achieves more complete immunological reconstitution than a single lobe (Leuchars, personal communication). If the thymus graft donor is a CBA/H.T6T6 (hereafter referred to as T6T6) mouse, then the population of lymphocytes deriving from the graft can be distinguished by the pair of marker chromosomes they carry (Plate 3.1). When such a graft is implanted immediately after irradiation, 30 days later 90% of the PHA responsive population in the peripheral blood carry the marker chromosomes. Subsequently a substantial number of host Lac cells are processed by the thymus graft and enter the peripheral circulation (Doenhoff and Davies, 1971). These cells which are thought to derive from the bone marrow are of Lac genotype but phenotypically are T cells. If the graft is allowed to remain in situ for 100 days, the PHA responsive population is found to consist of Lac and T6T6 cells in an approximately 50 : 50 ratio (Doenhoff and

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Plate 3.1 Chromosome spreads from CBA/Lac and CBA/H.T6T6 mice. CBA/H.T6T6 mice show a distinctive pair of marker chromosomes.

Davies, 1971). To prevent such dilution of the T cell pool the thymus grafts are removed 30 days after implantation. This procedure stabilizes the T cell pool and prevents its dilution by Lac cells (Doenhoff et al., 1970).

2. PREPARATION OF CBA/LAC — CBA/H.T6T6 MOUSE RADIATION CHIMAERAS

(Fig. 3.1)

All mice used in these experiments were obtained from the Chester Beatty Research Institute, London. Male CBA/Lac mice were thymectomized at 7 weeks of age. Thymectomy was carried out according to the method described previously. 10 days later the mice were X-irradiated with 850 rads from a Marconi Irradiation Unit. Within 4 hours of irradiation they were reconstituted with an I.V. injection of 5×10^6 femoral bone marrow cells from Lac donors. 20 hours later the mice were grafted with two thymus lobes from T6T6 neonates. All thymus grafts were placed under the left kidney capsule. Thymus graft implantation was performed as described earlier. 30 days after grafting, the thymus grafts were removed. 20 days after graft removal the mice were ready for infection.

3. REMOVAL OF THYMUS GRAFTS

(Plates 3.2a and 3.2b)

Mice were anaesthetised with ether, and anaesthesia was maintained with Penthrane inhalation anaesthetic. Autoclips were removed with an Autoclip remover (Clay-Adams Inc., U.S.A.). The

Fig. 3.1

PREPARATION OF CBA/ Lac - CBA/H.T_6T_6 MOUSE RADIATION CHIMAERAS

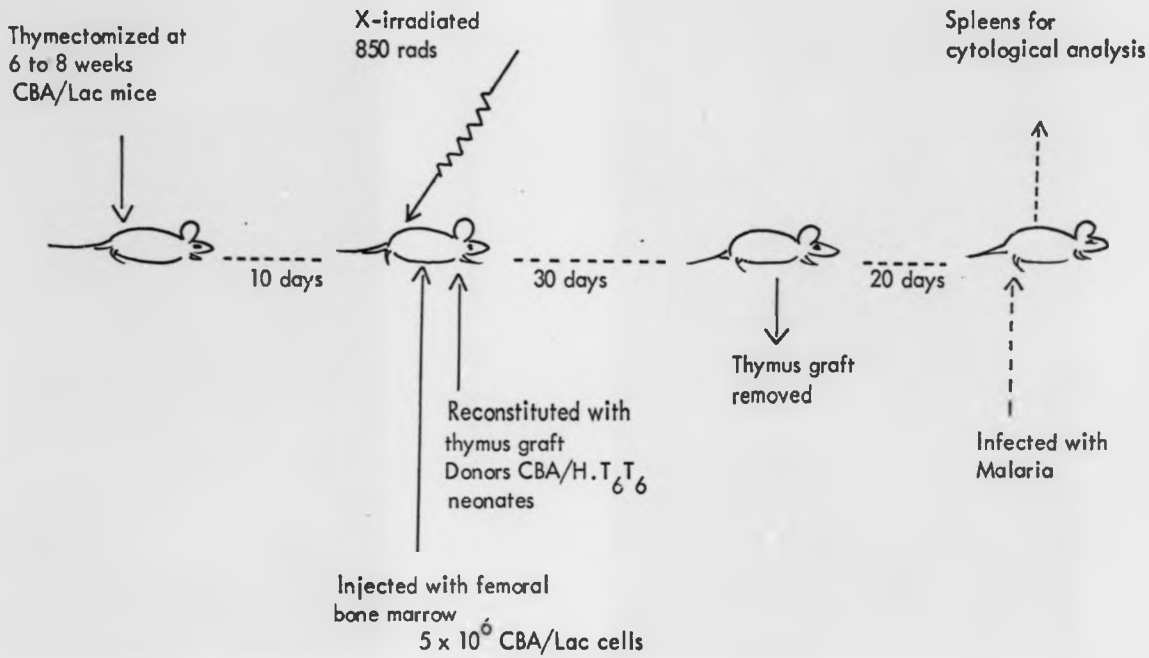


Plate 3.2a Thymus graft 30 days after implantation under the kidney capsule.

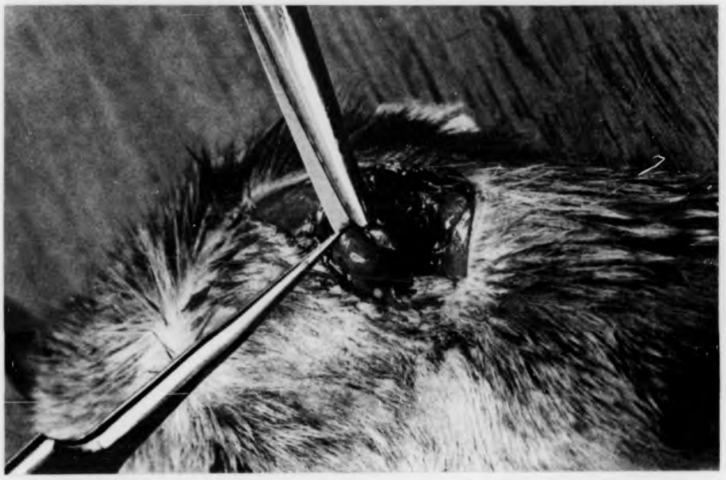
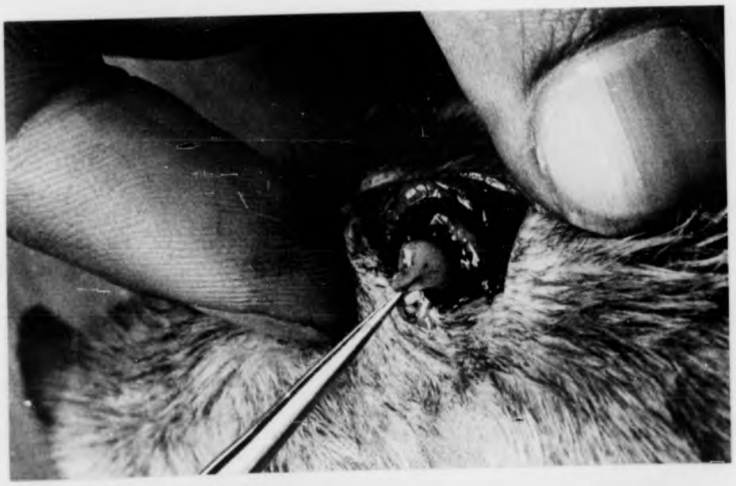


Plate 3.2b Thymus graft being "peeled off" kidney



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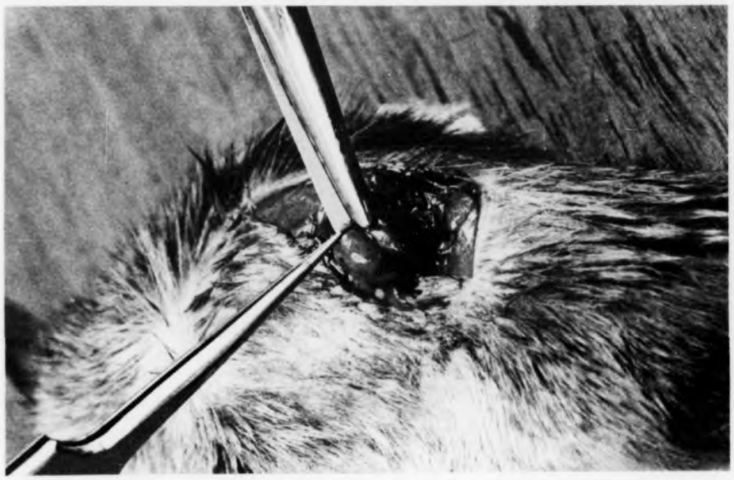

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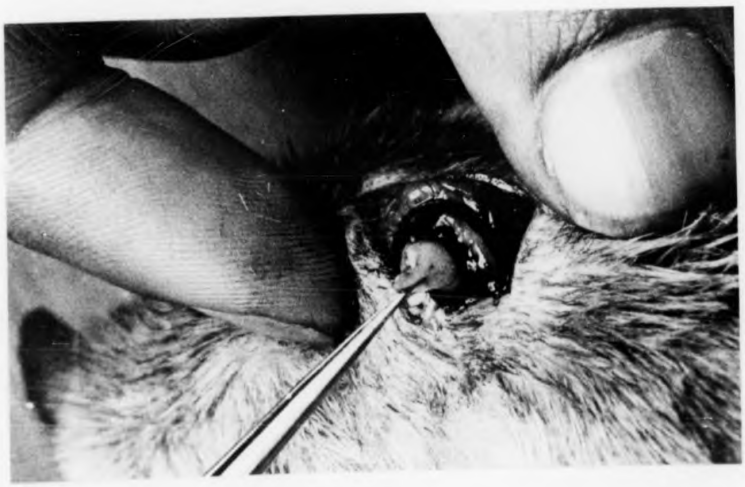

left flank was shaved, swabbed with 70% alcohol, incisions were made in the skin and body wall and the left kidney was exteriorized as for thymus grafting. The kidney capsule was raised with watchmaker forceps and an incision was made in the capsule above the thymus graft. With fine forceps the graft was peeled off the surface of the kidney. The kidney was pushed back into the peritoneal cavity and the incisions in the body wall and skin were closed with Autoclips.

4. INFECTION OF MICE

Mice were infected with 1×10^5 P. b. yoelii or P. b. berghei parasitized erythrocytes from infected donors. Parasitaemias were monitored in the usual manner.

5. CYTOLOGICAL TECHNIQUE

5.1 Colchicine pretreatment

Mice were injected I.P. with 0.3 ml of a stock solution of Colchicine at various intervals of time after infection. The stock solution was prepared by dissolving 10 mg Colchicine (Koch Light Laboratories, U.K.) in 25 ml of normal saline and was stored at $-70^{\circ}C$. The alkaloid Colchicine (prepared from the Autumn crocus) inhibits spindle formation and hence arrests cell division at metaphase.

5.2 Preparation of cell suspensions

1 to $1\frac{1}{2}$ hours after treatment with Colchicine the mice were killed by cervical dislocation, the spleens removed and placed in

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1 to $1\frac{1}{2}$ hours after treatment with Colchicine the mice were killed by cervical dislocation, the spleens removed and placed in

chilled T.C.199 medium (Wellcome). The spleens were cut into small fragments, passed through fine stainless steel sieves, and the cell suspension in 199 medium was transferred into small disposable polystyrene tubes. Each tube contained 1.5 to 2.0 ml of cell suspension at a concentration of 2 to 10×10^6 cells/ml. All further processing was carried out in these tubes.

5.3 Hypotonic treatment

The spleen cell suspension was centrifuged at 45g for 7 min, the supernatant decanted and the cells resuspended in 2.0 to 2.5 ml of fresh 1% Trisodium Citrate solution. After 15 to 30 min the cells were spun once more at 45g for 7 min.

5.4 Fixation

After hypotonic treatment, the citrate solution was decanted and a few drops of the fixative - a 3 : 1 solution of ethanol and acetic acid - were added, thus fixing the pellet of cells. After 2 to 3 min the fixative was removed, the tube flicked and the cells resuspended. The tube was now filled with fresh fixative, agitated gently, and left for 5 to 10 min. The cells were then spun down (45g x 7 min), resuspended in fresh fixative and placed in a refrigerator at $4^{\circ}C$ for 1 to 5 hours.

5.5 Chromosome spreading

The cell suspension was removed from the refrigerator, spun at 45g x 7 min, the supernatant was decanted completely and fresh fixative consisting of a 3 : 1 solution of methanol and acetic acid

was added. The fixative was added until a suitable cell density was reached.

One drop of the cell suspension was placed on a clean slide. (Slides were cleaned in a solution of 1% HCl in methyl alcohol). The drop was allowed to spread, and when Newton's rings became visible the excess fixative was blown off. A 100 watt lamp may be used at this stage to hasten evaporation of the fixative. Slides were allowed to dry and were then ready for staining.

5.6 Staining

Slides were stained according to the following procedure.

1. 10 min in 2% Orcein in 60% propionic acid.
2. 30 sec in methyl alcohol.
3. 30 sec in methyl alcohol.
4. 30 sec in absolute alcohol : Euparal essence.
5. 30 sec in Euparal essence.
6. Mount in Euparal vert.

5.7 Estimation of mitotic indices

Slides from each spleen were coded and examined on a Wild M-20 microscope. A minimum of 50 dividing cells were scored from each spleen, distinction being made between CBA/H.T6T6 cells derived from the thymus graft, and CBA/Lac cells derived from the bone marrow. The percentage of dividing T6T6 cells was used as an index of T cell activity. Total mitotic activity was estimated by counting the number of dividing to non

dividing cells and expressing it as a percentage.

6. HISTOLOGY

A part of each spleen used for cytological analysis was fixed in Carnoy's fixative and processed according to the histological methods described previously. Methyl green pyronin and haematoylin-eosin stained sections were prepared, coded and examined.

RESULTS

1. Parasitaemias

1.1 P.b. yoelii

The pattern of infection in CBA/Lac — CBA/H.T6T6 mouse radiation chimaeras infected with 1×10^5 parasitized erythrocytes is shown in Fig.3.2. Parasites could be detected in the peripheral blood by day 2. Peak parasitaemias were reached on day 10 at which stage 5 to 6% of the erythrocytes were infected. Parasites persisted in the peripheral blood until day 18 unlike in intact CBA mice which usually clear a similar inoculum in 15 to 16 days.

1.2 P. b. berghei

Infection of radiation chimaeras with 1×10^5 P. b. berghei parasitized erythrocytes resulted in an infection essentially similar to that seen in intact CBA mice. Over the first 10 days the parasitaemia was comparable to that observed in a P. b. yoelii infection, thereafter unlike P. b. yoelii infections, the parasitaemia increased

steadily and proved fatal in all mice by day 23 (Fig. 3.4).

2. CYTOLOGICAL ANALYSIS

2.1 P. b. yoelii

T cell reactivity (Fig. 3.2 and Table 3.1)

In uninfected mice 3 to 4% of the dividing cells in the spleen were T cells. Mice infected with P. b. yoelii showed a very rapid increase in the number of dividing T cells in the spleen. By day 4, 53.06% of the dividing population were those carrying the marker chromosomes. At this stage the mean percentage parasitaemia was < 1%. The proportion of dividing T cells remained at a high level - 40 to 50% - until day 13 at which time 52.50% of the cells in mitosis were of thymic origin. After day 13 there was a dramatic fall in T cell activity, and on days 18 and 25 of infection, when the parasite had been eliminated from the peripheral blood, only 2 to 3% of the dividing cells scored were of thymic origin.

Total mitotic activity (Fig. 3.3)

The number of dividing cells in the spleen increased rapidly until day 9 of infection, at which time peak activity was observed. After this there was a gradual decrease in mitotic activity, but even on day 25, 1 to 2% of the cells were dividing compared with 0.2% in uninfected controls.

2.2 P. b. berghei

T cell reactivity (Fig. 3.4 and Table 3.2)

P. b. berghei elicited a relatively weak T cell response in

Fig. 3.2 Mean percentage of thymus derived cells dividing in the spleen and mean percentage parasitaemias in CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras infected with P.b. yoelii.

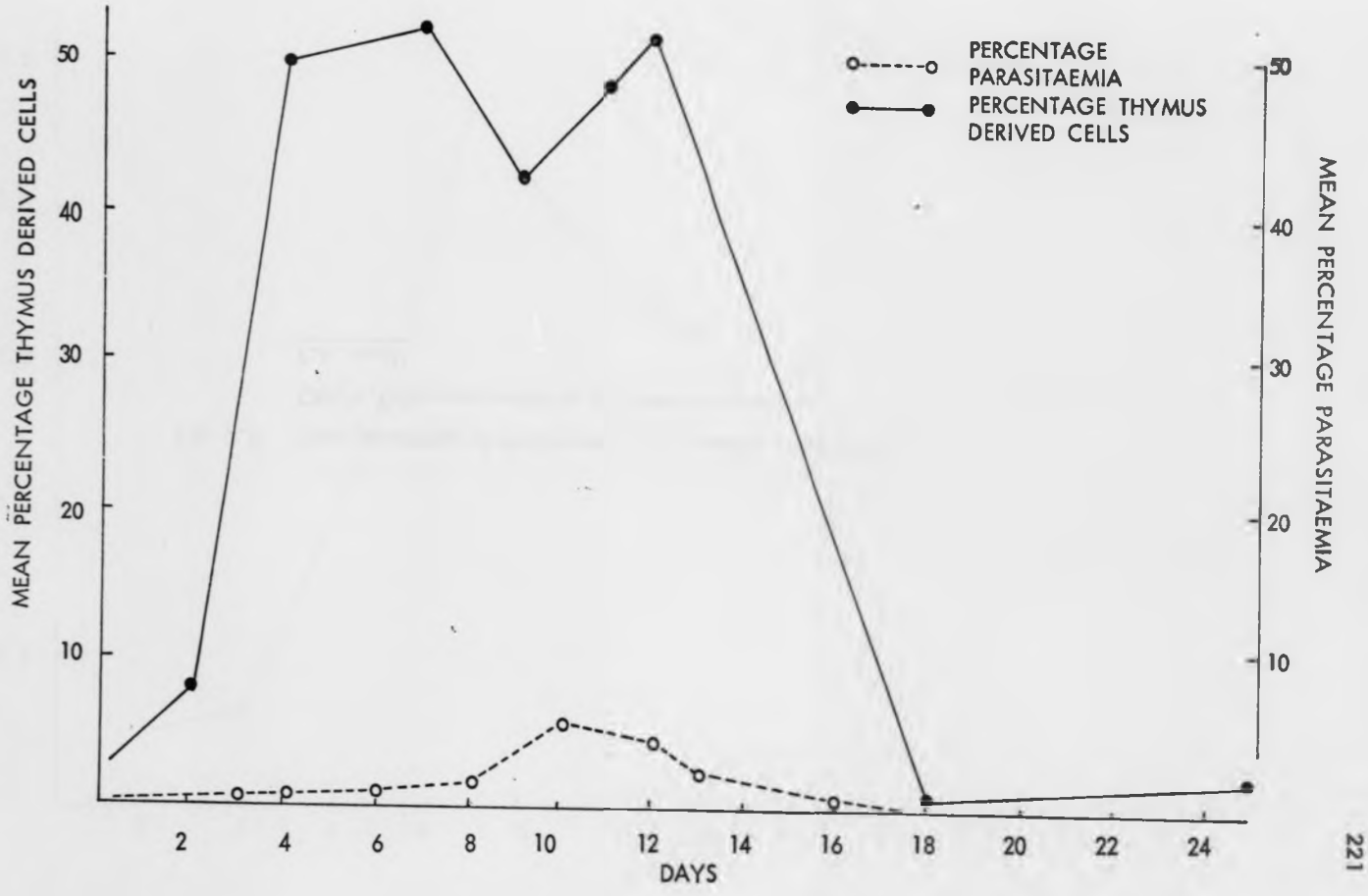

MEAN PERCENTAGE THYMUS DERIVED CELLS
MEAN PERCENTAGE PARASITAEMIA
PERCENTAGE PARASITAEMIA
PERCENTAGE THYMUS DERIVED CELLS
DAYS
10
20
30
40
50
2
4
6
8
10
12
14
16
18
20
22
24

Fig. 3.3 Mean percentage of dividing cells in the spleens of CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras infected with *P.b. yoelii*.

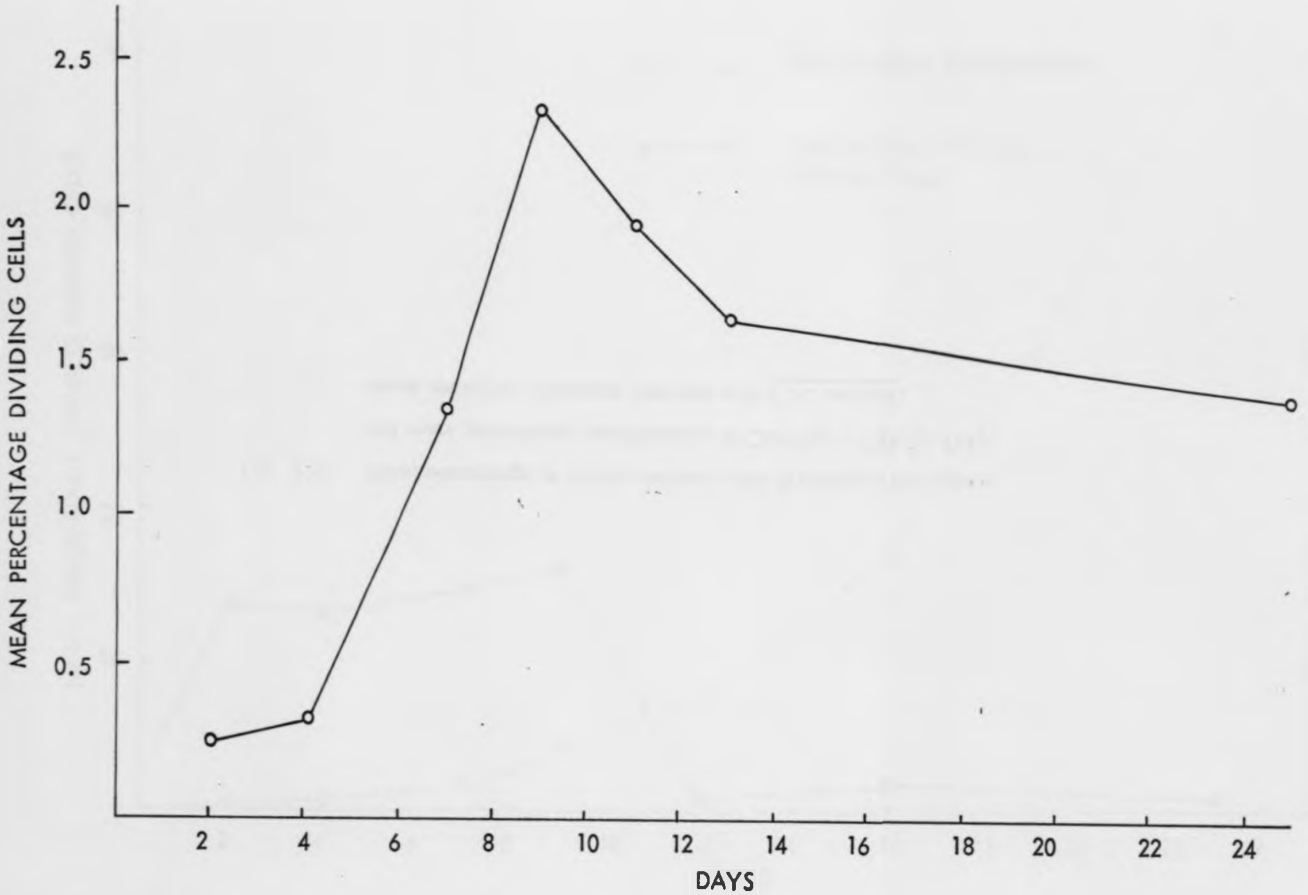
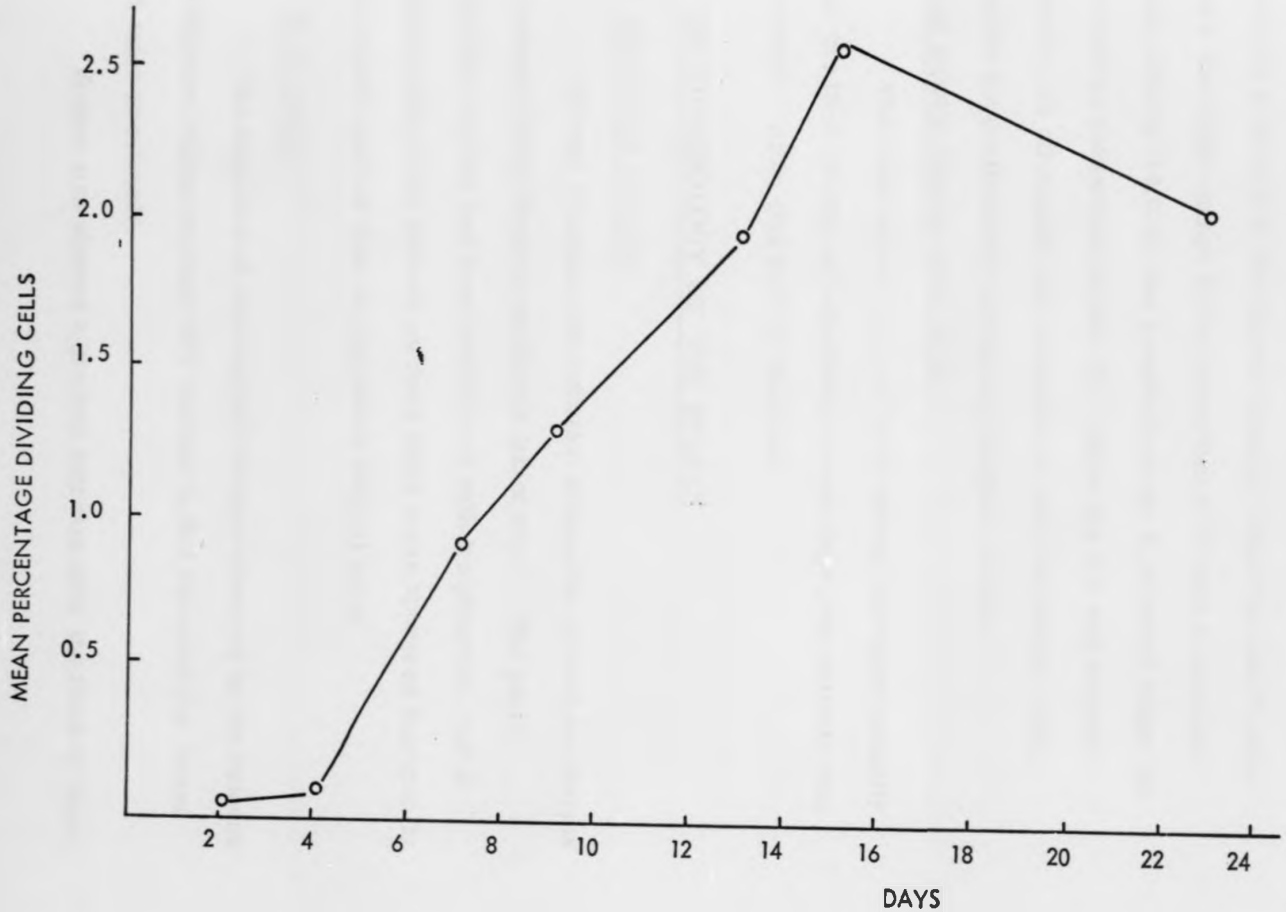

MEAN PERCENTAGE DIVIDING CELLS
2.5
2.0
1.5
1.0
0.5
2
4
6
8
10
12
14
16
18
20
22
24
DAYS

Fig. 3.4 Mean percentage of thymus derived cells dividing in the spleen and mean percentage parasitaemias in CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras infected with P.b. berghei.

MEAN PERCENTAGE THYMUS DERIVED CELLS
50
40
30
20
10
2
4
6
8
10

PERCENTAGE PARASITAEMIA
PERCENTAGE THYMUS DERIVED CELLS
MEAN PERCENTAGE PARASITAEMIA
50
40
30
20
10
12
14
16
18
20
22
24
DAYS

Fig. 3.5 Mean percentage dividing cells in the spleens of CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras infected with P.b. berghei.


MEAN PERCENTAGE DIVIDING CELLS
2.5
2.0
1.5
1.0
0.5
2
4
6
8
10
12
14
16
18
20
22
24
DAYS

mouse radiation chimaeras. On day 2 of infection the percentage of dividing T cells had increased from 3.45% in the uninfected controls to 13.51% in the infected group. Over the next 7 days there was little change in the proportion of T cells in mitosis. Peak activity (14.63%) was recorded on day 9, at which stage the percentage parasitaemia was 4%. After day 9 T cell mitotic activity fell off rapidly and remained at very low levels < 3%, despite the continuously increasing antigenic burden.

Total mitotic activity (Fig. 3.5)

The total mitotic activity in the spleen increased steadily over the first 16 days of infection at which stage peak activity was recorded. After this activity declined.

3. HISTOPATHOLOGY OF THE SPLEEN

3.1 Uninfected controls

Spleens of uninfected radiation chimaeras showed no obvious differences from those of uninfected intact mice. The peri-arteriolar regions had been repopulated with lymphocytes, but in haematoxylin-eosin stained sections these areas appeared marginally less densely packed than in completely normal mice.

3.2 P. b. yoelii

The sequence of histological changes observed in the spleens of radiation chimaeras was very similar to that recorded for intact CBA mice previously.

Spleen size showed a gradual increase over the first 11 days

of infection. After day 11, there was a gradual decrease in the splenomegaly observed. The maximum spleen weight recorded on day 11 was significantly less than that recorded in intact CBA mice at a comparable stage of infection.

White pulp

1. A few pyroninophilic cells accumulated in the periarteriolar zones from day 4 onwards. By day 7 these cells formed a conspicuous cuff around the central arteriole, and by day 11 the pyroninophilia in this region was maximal. After day 11 this response receded gradually, but even on day 25 some pyroninophilic cells were present around the central arteriole.
2. Germinal centres which were present at the edge of the follicles enlarged and showed increasing activity as the infection progressed. They contained pyroninophilic blast cells, macrophages, cell debris and dividing cells. Maximum activity was observed on day 25, at which time the germinal centres were very large and conspicuous. Although the germinal centre response paralleled that observed in intact mice, the kinetics of the response appeared to be marginally slower in the radiation chimaeras.

Red pulp

Much haematopoietic activity and pyroninophilia were evident as the infection progressed. After the peak of the infection activity in the red pulp waned gradually.

3.3 P. b. berghei

Spleen weight showed a gradual increase until day 16, after

which no further increase was observed.

White pulp

1. By day 4 there was a rapid infiltration of the periarteriolar region with pyroninophilic cells. The predominant cell type was a pyroninophilic 'lymphoid' cell, but a few mature plasma cells were also detected. This pyroninophilia increased rapidly and by day 12 was quite extensive and dominated the white pulp segments. The pyroninophilic cells formed large confluent zones and 80 to 90% of these cells appeared to be indeterminate pyroninophilic cells. This response seemed maximal on day 16 after which there was no significant change.
2. Germinal centre activity was not pronounced during the infection. Over the first 10 days germinal centres were present around the edges of the follicles and appeared to contain pyroninophilic elements, but they were few in number and were not well developed. After day 12 the germinal centres appeared quite small and inconspicuous; they were very poorly delineated and quiescent during the latter stages of the infection.

Red pulp

Progressive increase in red pulp activity could be seen as the infection progressed. By day 7 it was heavily infiltrated with pyronin positive cells and this trend continued throughout infection. Much haemozoin pigment was deposited in the red pulp.

DISCUSSION

Before discussing the significance of the experiments described, it is worth considering certain limitations inherent in the use of mouse radiation chimaeras. These radiation chimaeras do not regain a completely normal level of immunocompetence compared to unirradiated control mice (Davies et al., 1969a), and this is thought to be due to a partially irreparable radiation induced defect (Howard et al., 1971). This slightly impaired immune reactivity is probably reflected in the extended period of parasitaemia observed in P. b. yoelii infected radiation chimaeras.

The radiation chimaeras used in the present set of experiments had their thymus grafts removed 30 days after implantation so as to prevent progressive dilution of the marked T cell population derived from the graft by unmarked T cells. Recently, there has been increasing evidence that adult thymectomy by itself causes certain immunological and cellular changes (Bach et al., 1971; Raff and Cantor, 1971; Mosier and Cantor, 1971; Kerbel and Eidinger, 1972) including the loss of a short lived non-recirculating sub population of T cells (T_1 cells) found mainly in the spleen (Raff and Cantor, 1971). The possibility therefore exists that thymus graft removal could have a similar effect although there is no direct information available on this at present.

The experiments reported in this chapter showed that P. b. yoelii provoked a rapid and sustained burst of T cell mitosis, which declined once the infection was brought under control.

P. b. berghei on the other hand, induced a relatively feeble T cell response which was not sustained. In fact, the response failed despite the increasing parasite burden long before death of the animal due to the overwhelming parasitaemia. However, in both P. b. yoelii and P. b. berghei infections, after the decline of T cell activity, there was still considerable mitosis of the "unmarked" bone marrow derived cell population.

The precise biological significance of T cell mitosis is uncertain, but it is thought to reflect the immunological reactivity of the T cell pool. The two parasites P. b. yoelii and P. b. berghei show a fundamental difference in their ability to elicit such T cell mitotic activity. Further, while P. b. yoelii infections in the CBA mouse are non fatal, - the host mounting an effective immunological response against the parasite - P. b. berghei infections are always fatal in the CBA mouse within 25 days. The results of the present experiments therefore suggest that an important relationship possibly exists between T cell mitosis and the pathogenesis of Malaria.

Histopathological examination of the spleens of intact CBA mice and of radiation chimaeras, showed that while P. b. yoelii infections provoked a marked and sustained response in the germinal centres, in P. b. berghei infections the germinal centre response was relatively weak. On day 23, when the dominant feature of the P. b. yoelii infected spleens was the hyperactive germinal centres, in P. b. berghei infected mice, at a comparable time, the germinal

centres were relatively rudimentary in development. In fact, the poor germinal centre response observed in P. b. berghei infected mice was reminiscent of the response seen in T cell deprived mice infected with P. b. yoelii.

It would thus appear that a vigorous germinal centre response is found only in situations where a strong protective response is elicited, and it seems likely that it is the main site of production of protective antibodies. In the absence of a T cell population or in situations where the T cells fail to respond adequately, the germinal centre response seems to be seriously impaired.

Precisely why these two parasites evoke such fundamentally different responses is uncertain, but it is possible that they differ widely in their immunogenicity. P. b. yoelii is possibly strongly immunogenic and hence elicits a vigorous response, while P. b. berghei being less immunogenic provokes a weak response. In addition to a basic difference in immunogenicity, there are a number of other mechanisms which might underly the feeble T cell response and its subsequent failure in P. b. berghei infections. The parasite could affect the T cell pool either via the production of immuno-suppressive "factors" such as toxins, or exert a more direct effect on T cell reactivity. It has been shown that when a mouse is repeatedly injected with sheep red blood cells, after some time the T cell response fails due to the induction of some form of immunological paralysis (Gershon et al., 1968). It is not unlikely that the

constantly increasing parasite burden could induce a similar defect.

The considerable antigenic load could affect the integrity of the immune system in other ways as well. Kerbel (1974) has recently discussed the possibility that the inability of the T cell pool to respond effectively in some tumour bearing hosts could be due to the inimical effects of antigen-antibody complexes on the functional potential of T cells. Activated T cells have been shown to bear a receptor for the Fc portion of immunoglobulin when the immunoglobulin is complexed to specific antigen (Yoshida and Andersson, 1972), and it has been suggested that inhibitory complexes could exert their effects on T cells via such Fc receptors. Kerbel further suggests that the failure of the T cell mitotic response in CBA mice to an implanted allogenic tumour (Weston et al., 1972) could have been due to such a mechanism. In the parasite situation too it is known that large amounts of antigen and antibody exist together with activated T cells, hence the possibility that the inhibition of T cell mitotic activity observed in P. b. berghei infection might be due to such inhibitory complexes cannot be overlooked.

It was shown previously that P. b. berghei infections resulted in elevated steroid levels and marked thymic involution. The result of the present experiment suggests that such changes are probably a consequence of the failure of the T cell response.

Table 3.1 Cytological analysis of the spleens of CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras at various times after infection with P. b. yoelii

Day of infection	Total no. of cells scored	No. of CBA/H.T6T6 cells	No. of CBA/Lac cells	Percentage CBA/H.T6T6 cells
0	174	6	168	3.45
2	162	12	150	7.40
4	196	104	92	53.06
7	240	126	114	52.50
9	168	72	96	42.85
11	180	87	93	48.33
13	240	126	114	52.50
18	196	4	192	2.17
25	168	4	164	2.38

Table 3.2 Cytological analysis of the spleens of CBA/Lac - CBA/H.T6T6 mouse radiation chimaeras at various times after infection with P. b. berghei

Day of infection	Total no. of cells scored	No. of CBA/H.T6T6 cells	No. of CBA/Lac cells	Percentage CBA/H.T6T6 cells
0	174	6	168	3.45
2	116	16	100	13.79
4	222	30	192	13.51
7	288	40	248	13.88
9	328	48	280	14.63
12	180	2	178	1.11
16	210	6	204	2.85
23	222	3	219	1.35

CHAPTER 4

THE FUNCTIONAL STATUS OF THE LYMPHOCYTE POOL

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SECTION I. THE RESPONSE TO PHYTOHAEMAGGLUTININ AND BACTERIAL LIPOPOLYSACCHARIDE IN VITRO

INTRODUCTION

Malarial infections are known to depress the host's immune response to certain other antigens (see Chapter 1 for review). Although such depression has been demonstrated in a number of experimental situations, the mechanism by which malaria induces a state of reduced responsiveness remains unclear.

The characteristics of the immunodepression observed suggest that one or more of the cell types involved in the immune response may be affected. Histopathological studies of the spleen (reported earlier), showed that there were a number of cellular changes taking place, including an infiltration of the 'thymus dependent' areas with indeterminate pyroninophilic cells and some plasma cells which might have been contributory to the immunodepression observed. The present series of experiments was an attempt to define such changes in the lymphocyte population more precisely.

In these experiments the functional activity of the T and B lymphocyte populations were assayed in vitro, by studying their responsiveness to the polyclonal mitogens Phytohaemagglutinin (PHA) and bacterial Lipopolysaccharide (LPS). The state of the T cell pool was also assessed in vivo by examining the response to the skin sensitizing agent Oxazolone. These experiments are reported in section II of this chapter.

PHA, under appropriate culture conditions, is a specific T cell mitogen. That PHA preferentially stimulates mouse T cells was first demonstrated by Doenhoff (1971), who showed with the use of chromosome markers that virtually all blood lymphocytes responding to PHA were of thymic origin. More recently there have been reports that under certain special conditions PHA can also stimulate B cells. For instance, if PHA is not present in a soluble form but covalently bound to a solid matrix, stimulation of substantial numbers of B cells has been shown to occur (Greaves et al. , 1972; Andersson et al., 1972). Also mouse B cells appear to be stimulated if the period of culture is prolonged (Piguet and Vassali, 1972). Further, Phillips and Roitt (1973) claimed that human B cells were capable of responding directly to soluble PHA, but other workers (Greaves et al., 1974) have not been able to confirm this observation.

Bacterial lipopolysaccharide (LPS) on the other hand specifically stimulates mouse B cells (Gery et al., 1972; Andersson et al., 1972; Peavy et al., 1972; Greaves and Janossy, 1972; Doenhoff et al., 1974). Although the mitogenic effects of LPS on mouse B cells have not been extensively investigated, as yet, it seems likely that LPS acts only on a sub population of B cells (Gery et al., 1972; Melchers, personal communication). There is little evidence that LPS stimulates T cells although Piguet and Vassali (1973) claimed that a small number of T cells respond.

MATERIALS AND METHODS

1. ANIMALS

Male mice of the CBA/Lac and CBA/H.T6T6 strains were used in these experiments.

2. T CELL DEPRIVATION

T cell deprivation was carried out as described previously. CBA/Lac mice were thymectomized at 8 weeks, subjected to 850 rads X-irradiation 10 days later and were reconstituted haematopoietically with 5×10^6 bone-marrow cells from Lac donors, within 4 hours of X-irradiation.

3. INFECTION OF MICE AND PARASITAEMIAS

The parasites used in these experiments were P. b. yoelii and P. b. berghei. Infective inocula were prepared as described previously. Percentage parasitaemias were estimated from Giemsa stained thin blood films.

4. CULTURE MEDIA AND MITOGENS

Materials required for preparing the culture medium:

i. RPMI medium 1640 (Biocult Labs.)

ii. Glutamine 200 mM (Flow Laboratories)

iii. Foetal calf serum (Flow Laboratories).

100 ml desiccated foetal calf serum was reconstituted with 100 ml sterile distilled water, inactivated at $56^{\circ}C$ for 30 min and stored in aliquots of 2.75 ml at $-20^{\circ}C$.

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iv. Antibiotics

Penbritin (Penicillin) and Orbenin (Streptomycin) from Beecham Research Laboratories.

250 mg of each antibiotic were dissolved in the same 20 ml of sterile distilled water and 0.5 ml volumes of this solution stored at -20°C.

The culture medium consisted of

0.40 ml Glutamine

0.25 ml stock solution of antibiotics

2.75 ml reconstituted foetal calf serum

and was made up to 25 ml with RPMI 1640.

Mitogens

a) Phytohaemagglutinin (PHA), purified (Wellcome). 2 mg were dissolved in 5 ml sterile distilled water and stored at -20°C. For use it was diluted 1 : 16 with phosphate buffered saline and 25 µl were added to each culture.

b) Lipopolysaccharide from E. coli (LPS) (Difco). LPS was dissolved in sterile distilled water to give a concentration of 1 mg/ml and was stored frozen at -20°C. 25 µl of this stock solution was added to each culture.

5. PROCEDURE FOR SETTING UP CULTURES

Mice were killed by cervical dislocation. Their spleens

were removed aseptically and placed in chilled RPMI medium. The cells were teased out in culture medium, washed once and resuspended in fresh culture medium. Nucleated cell counts of each cell suspension prepared were carried out on a model D Coulter Counter (Coulter Electronics), after which the cell concentration of each suspension was adjusted to 2×10^6 cells/ml. Spleens from untreated T6T6 mice and experimental Lac mice were processed in this manner.

0.5 ml portions of a cell suspension prepared from an untreated T6T6 mouse and adjusted to a concentration of 2×10^6 cells/ml were placed in a series of culture tubes (flat based vials, Sterilin Ltd.) 0.5 ml of cell suspension from individual experimental Lac mice were added to each tube and the cells well mixed. The mixture of T6T6 and Lac cells were now divided between 2 culture tubes, 0.5 ml/tube, and the appropriate mitogen was added to each tube. Cultures were incubated in 8% CO_2 and 82% N_2 at 37^oC.

6. HARVESTING OF CULTURES

Metaphase arrest for cytological analysis was achieved by adding Colcemid (Ciba) to the cultures on the third day of culture at a concentration of 10^{-7}M, 16 hours prior to harvesting. Metaphase spreads were prepared by the method of Ford (1966). The details of the cytological technique were as described in the previous section, but in the present series of experiments Colcemid was used instead of Colchicine.

7. ESTIMATION OF LAC : T6T6 RATIO

75 to 100 metaphases were scored from each culture and the ratio of Lac : T6T6 cells determined. This ratio was used as an index of responsiveness for each sample of 10^6 cells.

8. METHOD OF QUANTITATION EMPLOYED

The method used to quantitate the PHA and LPS responsive populations was that of Doenhoff (1971), and is based on a cytological analysis of cells derived from two syngeneic strains of CBA mice - CBA/Lac and CBA/H.T6T6. Cells of the T6T6 strain carry a pair of minute chromosomes by means of which they may be distinguished at metaphase.

In this method, a known number of cells from untreated mice of one strain is mixed with an equal number of cells from mice of the second strain which have been experimentally manipulated. The mixture of the two cell types is cultured with mitogen for 3 days, when, following metaphase arrest with Colcemid, the cells are fixed and subjected to cytological analysis. The ratio of the dividing cells from the two sources is determined and the result so obtained gives the relative concentration of mitogen responsive cells in the experimental animal. For instance, if both Lac and T6T6 cells were derived from completely normal mice, an equal number of T6T6 and Lac cells would respond to a given mitogen and the Lac : T6T6 ratio would be 1. If the test Lac mice had fewer cells responding to the mitogen, the Lac : T6T6 ratio would be < 1.

The Lac : T6T6 ratio which is an index of the proportion of

cells responding to a given mitogen in culture was designated the index of responsiveness/ 10^6 cells. (A culture consisted of 10^6 cells.) In order to estimate the degree of responsiveness/ spleen, the index/ 10^6 cells was multiplied by the mean number of nucleated cells in the spleen from which the cultured cells were derived. This was termed the index of responsiveness/ spleen.

A disadvantage of this method of quantitation is that its use is limited to the CBA/Lac and CBA/H.T6T6 mouse strains.

9. DATA ANALYSIS

All estimates of mitogen responsiveness were made on 5 cultures derived from individual mice. Mean values and standard errors (S.E.) were calculated on an Olivetti 'Programma 101' desk computer. The significance of differences between groups was calculated by Student's t test.

EXPERIMENTS

The data presented in this section consists of the results of three experiments.

1. Groups of intact and T-cell deprived CBA/Lac mice were infected with 1×10^5 P. b. yoelii parasitized erythrocytes. On day 11 of infection the intact and "deprived" mice were sacrificed together with the appropriate uninfected controls and the spleen cell suspensions derived from individual mice were set up in culture with PHA and LPS.

2. A group of intact CBA/Lac mice were infected with 1×10^5 P. b. yoelii parasitized erythrocytes. 30 days after infection, the spleens of these "recovered" mice and groups of normal uninfected controls were assayed as in experiment 1.

3. Intact CBA/Lac mice were infected with 1×10^5 P. b. berghei parasitized erythrocytes. On day 15 the spleens from these infected mice and uninfected controls were set up in culture with PHA and LPS.

There was very close agreement in the proportion of cells from normal uninfected controls responding to PHA and LPS in the three experiments. Hence the controls in experiments 2 and 3 are not shown in the results.

RESULTS

1. THE RESPONSE TO PHYTOHAEMAGGLUTININ (PHA)

(Figs. 4.1a, 4.1b and Table 4.1)

1.1 Intact mice infected with P. b. yoelii

Uninfected mice showed the expected PHA index which approached 1. In the infected group (on day 11), there was a significant decrease in the proportion of PHA responsive cells, the PHA index/ 10^6 cells being 0.29 compared to 0.97 in the uninfected controls ($p < 0.001$). By day 30 there appeared to be recovery of the PHA responsive pool, the PHA index/ 10^6 cells (0.71) not being significantly different from the controls.

Although there had been a drop in the proportion of cells responding, there was no decrease in the absolute number of PHA responsive cells/ spleen. By day 11 of infection, cell numbers in the spleen had increased from 1.53 to 12.22×10^8 and as a result of this splenomegaly, the PHA responsive index/ spleen in the infected group was 3.42×10^8 compared to 1.51×10^8 in the controls ($p < 0.001$). On day 30 the PHA index/ spleen was still higher than in the controls but the difference was not significant.

1.2 Deprived mice infected with P. b. yoelii

T cell deprived mice have been shown to have a small residual population of T cells variously estimated at between 5 and 10% of the normal T cell pool (Doenhoff,1971; Raff, 1971). The "deprived" mice used in these experiments had a PHA responsive index which was approximately 10% of that in the intact mice. On day 11 of infection the PHA responsive index/ 10^6 cells was 0.12 compared with 0.14 in the uninfected "deprived" mice ($p < 0.05$).

When the PHA responsive index was calculated on a per spleen basis, the index was found to increase from 0.19×10^8 in the uninfected group to 0.41×10^8 in the infected group. Hence on day 11 of infection, the PHA index/ spleen in the deprived group was approximately 1/10th the value obtained in intact mice at a comparable stage of infection.

1.3 Intact mice infected with P. b. berghei

P. b. berghei infections in intact CBA mice proved fatal

Fig. 4.1a The index of PHA responsiveness per 10^6 spleen cells $\pm$ 1 S.E.

Fig. 4.1b The index of PHA responsiveness per spleen $\pm$ 1 S.E.

S.E.

INDEX OF PHA RESPONSIVENESS/SPLEEN x 10^8

0.5 1.0 1.5 2.0 2.5 3.0

Intact Untreated

Intact Day 11 P.b. yoelii

Intact Day 30 P.b. yoelii

Deprived Untreated

Deprived Day 11 P.b. yoelii

Intact Day 15 P.b. berghei

n cells ± 1 S.E.

INDEX OF PHA RESPONSIVENESS/10^6 CELLS
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
3.5
Intact Untreated
Intact Day 11 P.b. yoelii
Intact Day 30 P.b.y
Deprived Untreated
Deprived Day 11 P.b. yoelii
Intact Day 15 P.b. berghei

within 20 to 25 days. Spleens were assayed on day 15 of infection when the mean percentage parasitaemia was 20%. A marked reduction in the proportion of PHA responsive cells was recorded; the PHA index/ 10^6 was 0.13 compared to 0.97 in the uninfected controls ($p < 0.001$), and approached the level found in T cell deprived mice.

The PHA index/ spleen also showed a significant reduction from 1.51×10^8 in the control to 0.74×10^8 in the P. b. berghei infected group - ($p < 0.05$), thus indicating that by day 15 of infection there had been a reduction in the absolute size of the PHA responsive pool.

2. THE RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE (LPS) (Figs. 4.2a, 4.2b and Table 4.2)

2.1 Intact CBA mice infected with P. b. yoelii

On day 11 of infection P. b. yoelii infected mice showed a significant decrease in the proportion of LPS responsive cells. The LPS index/ 10^6 cells having fallen from 0.85 in the uninfected controls to 0.30 in the infected group ($p < 0.005$). On day 30 the recovered mice still showed very significant depression of the response to LPS, the index/ 10^6 cells being 0.28 ($p < 0.001$).

The calculated index of LPS responsiveness/ spleen showed that despite the decrease in the proportion of LPS responsive cells, there was a considerable increase in the total number of cells responding to LPA on day 11 - the LPS index/ spleen being

Fig. 4.2a The index of LPS responsiveness per 10^6 spleen cells $\pm$ 1 S.E.

Fig. 4.2b The inde:: of LPS responsiveness per spleen $\pm$ 1 S.E.

1 S.E.
INDEX OF LPS RESPONSIVENESS/SPLEEN x 10^{α}
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Intact Untreated
Intact Day 11 P.b. yoelii
Intact Day 30 P.b yoelii
Deprived Untreated
Deprived Day 11 P.b.yoelii
Intact Day 15 P.b.berghei

n cells ± 1 S.E.

INDEX OF LPS RESPONSIVENESS/10^6 CELLS
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Intact Untreated
Intact Day 11 P.b.yoelii
Intact Day 30 P.b. yoelii
Deprived Untreated
Deprived Day 11 P.b.y
Intact Day 15 P.b. berghei

3.56×10^8 compared to 1.30×10^8 in the controls ($p < 0.05$). Recovered mice showed a drop in the LPS index/ spleen to 0.80×10^8.

2.2 Deprived mice infected with P. b. yoelii

Uninfected T cell deprived mice had more LPS responsive cells in the spleen than intact mice. The LPS index/ 10^6 cells was 1.20 in the deprived and 0.85 in the intact group ($p < 0.05$). Doenhoff (personal communication) has observed a similar level of endotoxin responsive cells in the spleens of "deprived" mice. On infection there was a slight decrease in the proportion of LPS responsive cells in the "deprived" spleens - but this was not statistically significant.

The LPS index/ spleen was 2.92×10^8 on day 11 of infection compared to 1.64×10^6 in uninfected deprived mice, and 3.56×10^8 in intact infected mice.

2.3 Intact CBA mice infected with P. b. berghei

P. b. berghei infections also caused a significant decrease in the proportion of LPS responsive cells to levels similar to that seen in P. b. yoelii infections. On day 15 the LPS index/ 10^6 cells was 0.26 compared to 0.85 in the controls ($p < 0.01$). As P. b. berghei caused less splenomegaly than P. b. yoelii, the calculated LPS index/ spleen (1.75×10^8) did not differ significantly from control levels (1.30×10^8).

DISCUSSION

All experiments reported here were carried out with spleen

cell populations. Although using the spleen is advantageous since the entire organ can be assayed, it must be remembered that the spleen is but a fraction of the entire lymphocyte pool, and changes in the spleen need not necessarily reflect the state of the peripheral blood, thoracic duct lymph or lymph nodes. Further, these populations were sampled only at certain selected points during these infections.

These experiments show that both P. b. yoelii and P. b. berghei infections cause a marked reduction in the proportion of PHA responsive cells (T cells) in the spleen. In P. b. berghei infections there appears to be a significant reduction in the overall size of the T cell pool. In P. b. yoelii infections, despite a decrease in the proportion of responsive cells, there appears to be a significant increase of the total number of PHA responsive cells in the spleen. This would suggest that the decrease in the proportion of responsive cells on day 11, is due to "dilution" of the responding population either due to an increase of non responding cells or due to functional inactivation of some of the responding cells.

Both parasites also caused a significant reduction in the proportion of LPS responsive (B) cells. In P. b. yoelii infected mice the LPS response remained depressed even on day 30, at which stage there had been significant recovery of the PHA responsive pool.

A number of factors probably contribute to the reduced immunological reactivity observed during these infections. An attempt will be made to analyse some of the mechanisms which might

be responsible for such altered reactivity in the T and B cell pools.

1. Both P. b. yoelii and P. b. berghei cause a gradual infiltration of the 'thymus dependent' areas in the spleen with an indeterminate pyroninophilic lymphoid cell. In P. b. yoelii infections this infiltration is greatest at the peak of the infection (days 10-12), a time at which maximum immunodepression is known to occur (Salaman et al., 1969; Greenwood et al., 1971). On recovery, this infiltration gradually recedes and by day 30 only a few of these cells are found cuffing the central arteriole. In P. b. berghei infections there is a similar progressive infiltration. By day 15 (the time at which spleens were assayed) these cells form an extensive confluent mass.

The infiltration of these T cell areas could be one of the factors which cause a decrease in the proportion of PHA responsive cells in these infections. The kinetics of the response correlating well with the level of depression observed. Such dilution of the T cell pool by apparently non-responsive elements could also be an important factor in the depressed humoral antibody responses to T-dependent antigens seen in these infections. P. b. yoelii infections are known to depress humoral antibody responses to sheep red blood cells (SRBC) (Salaman et al., 1969; Greenwood et al., 1971), heat aggregated gamma globulin (HGG) (Greenwood et al., 1971), and tetanus toxoid (Voller et al., 1972). P. b. berghei has also been shown to depress the plaque forming cell (PFC) response to SRBC (Whitemore, 1973).

A "crowding out" of T cells could lead to altered cell densities and cell relationships which could be particularly important in responses where cell collaboration is involved. Mosier (1969) has drawn attention to the fact that specific cell clusters are required for immune responses to T dependent antigens in vitro. It seems very likely that similar conditions may be needed in vivo. Hence changes which disrupt the functional integrity of T cell areas may limit an animal's ability to mount an effective response, particularly to thymus dependent antigens such as SRBC.

2. It was demonstrated previously that P. b. yoelii infected mice had a considerable number of T cells in mitosis. Such mitotic activity was maximal around the peak of the infection and returned to normal levels by day 25. It is conceivable that the depressed responsiveness to PHA and antigens such as SRBC seen during an active P. b. yoelii infection, might in part be due to the fact that a large number of antigen reactive cells are "preoccupied" responding to the parasite. A number of investigators (Radovich and Talmage, 1967; Eidinger et al., 1968; Brody and Siskind, 1969; Hunter et al., 1969) have shown that the administration of two different antigens, simultaneously or consecutively, results in diminished responsiveness to one of the antigens. This phenomenon has been termed antigenic competition. O'Toole and Davies (1971) found that simultaneous or consecutive injections of the same antigen (SRBC), also resulted in reduced responsiveness - a phenomenon which they termed "pre-emption" as the first injection of antigen

"appropriated" the animal's capacity to respond to the second injection of antigen. It has also been demonstrated that mice injected with SRBC show significantly depressed PHA responses (Adler et al., 1971). Further, Adler et al. (1971) concluded that the reduced proportion of PHA responsive cells in tumour bearing mice was probably a consequence of their temporary preoccupation with tumour specific antigens.

Hence, the decrease in the proportion of PHA reactive cells in the spleen during a P. b. yoelii infection may reflect further evidence of an intense T cell response to the parasite, rather than being indicative of a state of immunological deficiency in any absolute terms.

P. b. berghei on the other hand, induces only limited activity in the T cell pool. It seems unlikely, therefore, that pre-emption of the T cell pool would be the main reason for the immunodepression observed in this infection.

3. In the present study both P. b. yoelii and P. b. berghei infections were shown to cause a significant increase in plasma corticosterone levels. In P. b. yoelii infections the increase was temporary; in P. b. berghei elevated levels are probably maintained throughout the infection. (A level of 17.5µg/100 ml was recorded on day 15.) Also, by day 15 there was considerable depletion of the corticosteroid sensitive thymic cortex.

The lympholytic effects of corticosteroids are now well established. Their effects on the immunologically incompetent

thymic cortical lymphocytes has been well documented in mice (Blomgren and Andersson, 1969; Cohen and Claman, 1971). Large doses of cortisone acetate have also been shown to inhibit the PHA responsiveness of mouse spleen cells (Levine and Claman, 1970), and more recently steroids have also been shown to affect the recirculatory properties of mouse T cells (Cohen, 1972). Folch and Waksman (1974) have suggested that steroids may exert their effects via a population of suppressor T cells. They have shown that under stress the suppressor T cells of young rats are activated and are able to inhibit the T cell response to PHA.

4. The mechanisms discussed so far have been concerned mainly with changes in the T cell pool. Both infections also induce significant depression of the LPS response. Interpretation of these results is made difficult by the fact that the effects of mitogens on B cells have been incompletely characterised. However, the cells which respond to B cell mitogens such as LPS, PPD (Purified Protein Derivative) and poly A : U (polyadenylic-polyuridylic acid complex) are thought to be a sub population of small resting lymphocytes which differentiate on stimulation to antibody forming cells which secrete IgM (Melchars, personal communication; Melchars and Andersson, 1974). The depressed response to LPS observed in these infections might be due to the fact that the responsive cells have already been triggered off by the parasite along a differentiation pathway leading to the formation of antibody secreting cells which are incapable of responding to LPS. The histopathological picture

and the considerable mitosis in the non T cell pool even during the later stages of the infection would be consistent with such an interpretation. It must however be pointed out that malarial "toxins" produced by the parasite itself may be potent B cell mitogens which have the ability to stimulate resting B cells. Such "toxins" are likely to persist even after the elimination of the infection, and may be one of the reasons for the continued depression of the LPS response in recovered mice.

It can thus be seen that a number of factors may be involved in the diminished functional reactivity of the T and B cell pools seen in P. berghei infections. It seems likely that the part played by any single factor would depend on the nature and severity of the infection, the time at which the immunological status of the animal is assessed, and the type of test "system" used.

Table 4.1 The response to Phytohaemagglutinin (PHA)

Group	Lac : T6T6 ratio PHA index/10^6 cells ± S.E.	Mean no. of cells/spleen x10^6 ± S.E.	Calculated PHA index/spleen x10^6 ± S.E.
Intact, uninfected	0.97 ±0.09	1.53 ±0.05	1.51 ±0.17
Intact P. b. y. infected Day 11	0.29 ±0.04	12.22 ±0.98	3.42 ±0.24
Intact P. b. y. infected Day 30	0.71 ±0.07	2.83 ±0.10	2.02 ±0.15
Deprived, uninfected	0.14 ±0.01	1.35 ±0.04	0.19 ±0.02
Deprived P. b. y. infected Day 11	0.12 ±0.04	3.37 ±0.22	0.41 ±0.12
Intact P. b. b. infected Day 15	0.13 ±0.03	6.57 ±0.68	0.74 ±0.21

Table 4.2 The response to Bacterial Lipopolysaccharide (LPS)

Group	Lac : T6T6 ratio LPS index/10^6 cells ± S.E.	Mean no. of cells/spleen x 10^6 ± S.E.	Calculated LPS index/spleen x 10^6 ± S.E.
Intact, uninfected	0.85 ±0.03	1.53 ±0.05	1.30 ±0.07
Intact P. b. y. infected Day 11	0.30 ±0.09	12.22 ±0.98	3.56 ±0.83
Intact P. b. y. infected Day 30	0.28 ±0.02	2.83 ±0.10	0.80 ±0.06
Deprived, uninfected	1.20 ±0.04	1.35 ± 0.40	1.64 ±0.10
Deprived P. b. y. infected Day 11	0.88 ±0.16	3.37 ±0.22	2.92 ±0.11
Intact P. b. b. infected Day 15	0.26 ±0.03	6.57 ±0.68	1.75 ±0.08

SECTION II. THE RESPONSE TO OXAZOLONE

INTRODUCTION

The experiments reported here were carried out to investigate the functional state of the T cell pool *in vivo*, in mice infected with malaria. This was done by studying the effect of malarial infections on a specific cell mediated immune response - contact sensitivity to 2-phenyl-4-ethoxymethylene-oxazolone (Oxazolone).

It has been shown that contact sensitivity to oxazolone can be easily induced in normal mice (Asherson and Ptak, 1968; Ptak and Asherson, 1969; de Sousa and Parrott, 1969), but not in mice deprived of their T cell population, either as a result of neonatal thymectomy (de Sousa and Parrott, 1969) or adult thymectomy followed by x-irradiation and reconstitution with bone marrow (Parrott *et al.*, 1970), or in congenitally thymusless "nude" mice (Pritchard and Micklem, 1971).

As the degree of responsiveness to oxazolone in mice is dependent on their level of T cell competence, it provides a convenient method of assaying the status of this cell population during malarial infections.

MATERIALS AND METHODS

1. MICE

CBA/Lac mice and outbred T.O. Swiss mice were used in these experiments.

2. SENSITIZATION

Mice were sensitized by applying 0.1 ml of a 3% solution of Oxazolone in alcohol to the skin of the clipped abdomen.

3. RECALL RESPONSE

The sensitized and non sensitized controls were tested for contact sensitivity 6 to 7 days after sensitization, by placing one drop of 1% oxazolone dissolved in olive oil, on to both sides of the right and left ears. Ear thickness was measured with a dial gauge micrometer (Mercer Model 911411.003/3 Calliper, St Albans) by a method similar to that described by Asherson and Ptak (1968), at 4, 24, 48 and 72 hours after the application of the test solution. (The response in T.O. mice was not measured at 72 hours.) The results were expressed as the mean percentage increase in ear thickness. Tests for the significance of differences between groups were calculated by Student's t-test.

EXPERIMENTS AND RESULTS

1. THE RESPONSE TO OXAZOLONE

The response to oxazolone was studied in three experimental systems -

1. CBA/Lac mice infected with P. b. yoelii
2. CBA/Lac mice infected with P. b. berghei
3. T.O. mice during and after recovery from a P. b. yoelii infection.

All infections were initiated by the intraperitoneal inoculation of

1×10^5 parasitized erythrocytes.

1.1 CBA mice infected with P. b. yoelii

(Fig. 4.3 and Table 4.3)

These mice were sensitized on day 10 of infection together with a group of uninfected controls, and tested 6 days later. The control CBA mice showed the typical delayed type response - ear swelling being maximal at 24 hours, at which stage a 74.58% increase in ear thickness was recorded. The response subsided gradually. At 48 and 72 hours, 64.50 and 53.33 per cent increases in ear thickness were recorded. The CBA mice infected with P. b. yoelii showed no significant difference in responsiveness from the uninfected controls at 24, 48 and 72 hours.

1.2 CBA mice infected with P. b. berghei

(Fig. 4.4 and Table 4.4)

Mice were sensitized on day 7 of a P. b. berghei infection together with a group of uninfected controls. Both groups were tested 6 days later. Mice infected with P. b. berghei showed severe depression of the response to oxazolone at 24 and 48 hours compared to the controls ($p < 0.001$). At 72 hours the response was still significantly impaired ($p < 0.01$).

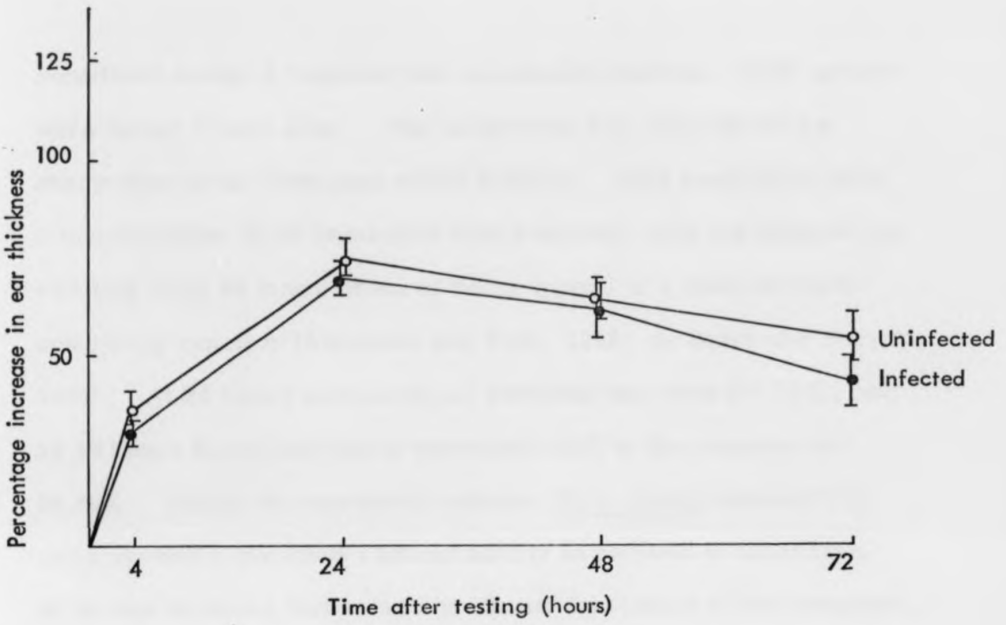
1.3 T.O. mice infected with P. b. yoelii

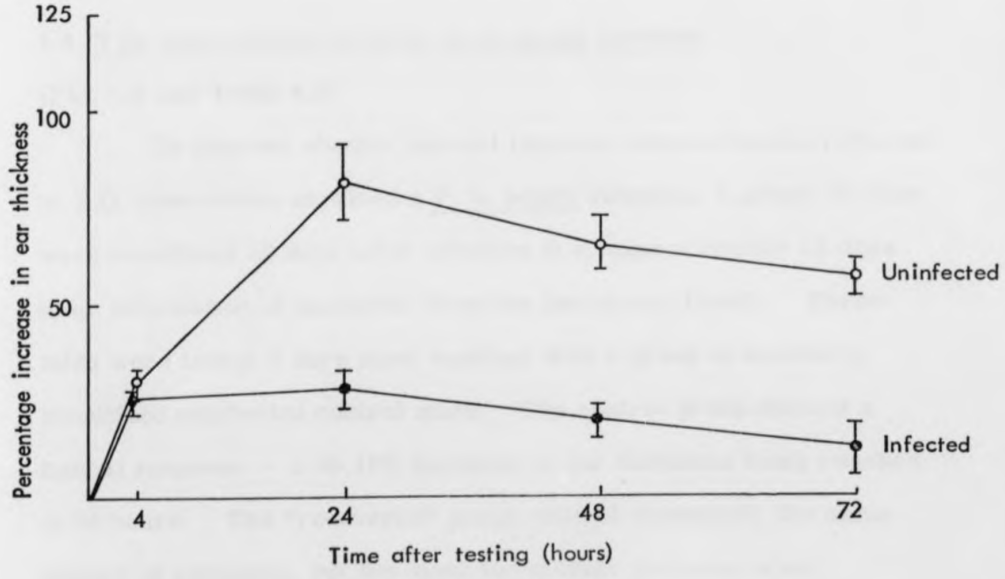
(Fig. 4.5 and Table 4.5)

P. b. yoelii produces an acute infection in outbred T.O. mice (see Chapter 1 for pattern of infection). Mice were

Fig 4.3 Mean percentage increase in ear thickness ± 1 S.E. in uninfected CBA mice and P.b. yoelii infected CBA mice sensitized with Oxazolone.

Fig. 4.4 Mean percentage increase in ear thickness ± 1 S.E. in uninfected CBA mice and P.b. berghei infected CBA mice sensitized with Oxazolone.


125
100
50
Percentage increase in ear thickness
Uninfected
Infected
4
24
48
72
Time after testing (hours)


125
100
50
Percentage increase in ear thickness
Uninfected
Infected
4
24
48
72
Time after testing (hours)

sensitized on day 8 together with uninfected controls. Both groups were tested 7 days later. The uninfected T.O. mice showed a sharp rise in ear thickness within 4 hours. This could have been a manifestation of an immediate type reaction; only the delayed ear swelling from 24 hours onwards being typical of a delayed hypersensitivity reaction (Asherson and Ptak, 1968; de Sousa and Parrott, 1969). At 24 hours maximum ear swelling was seen (97.75%), and by 48 hours there had been a substantial fall in the response to 54.46%. Unlike the untreated controls, P. b. yoelii infected T.O. mice showed a markedly reduced ability to respond to oxazolone. At 24 and 48 hours there was significant depression of the response, the mean increase in ear thickness being 38.70% ($p < 0.001$) and 29.25% ($p < 0.05$) respectively.

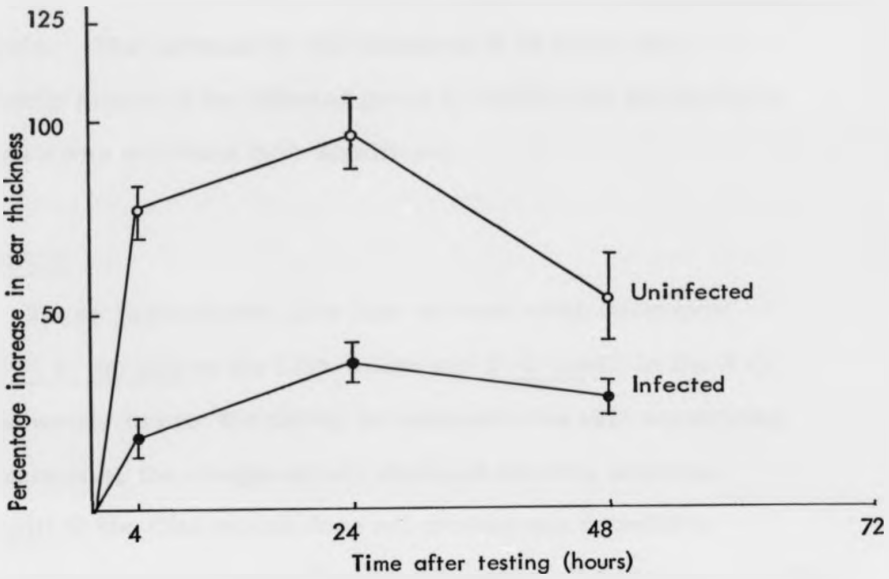
1.4 T.O. mice recovered from P. b. yoelii infection

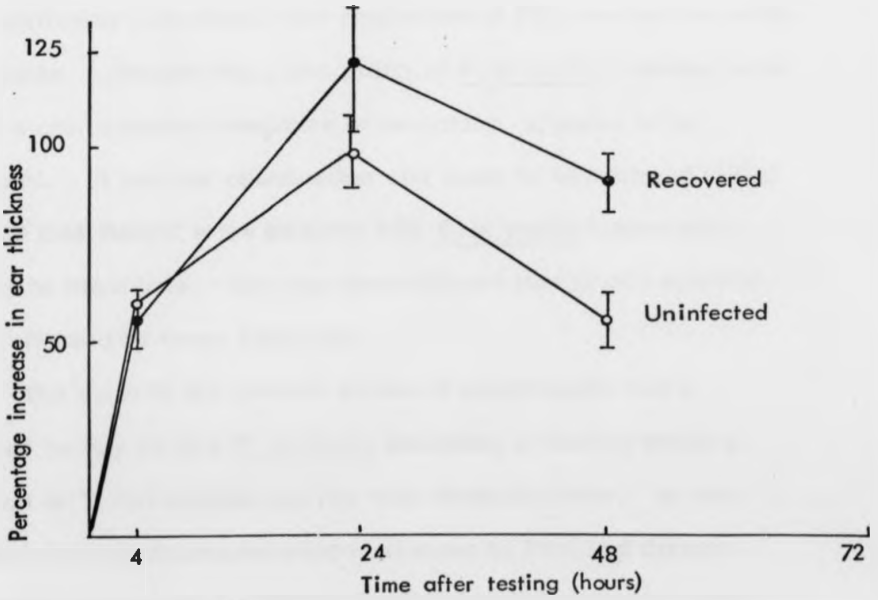
(Fig. 4.6 and Table 4.6)

To find out whether normal immune responsiveness returned to T.O. mice which survived a P. b. yoelii infection, a group of mice were sensitized 29 days after infection (i.e. approximately 12 days after elimination of parasites from the peripheral blood). These mice were tested 7 days later together with a group of similarly sensitized uninfected control mice. The control group showed a typical response - a 99.12% increase in ear thickness being reached at 24 hours. The "recovered" group showed essentially the same pattern of response, but the mean percentage increase in ear thickness in the recovered group at 24 and 48 hours was higher than

Fig. 4.5 Mean percentage increase in ear thickness ± 1S.E. in normal uninfected T.O. mice and P.b. yoelii infected T.O. mice sensitized with Oxazolone.

Fig. 4.6 Mean percentage increase in ear thickness ± 1 S.E. in normal uninfected T.O. mice and T.O. mice recovered from a P.b. yoelii infection.


125
100
50
Percentage increase in ear thickness
Uninfected
Infected
4
24
48
72
Time after testing (hours)


125
100
50
Percentage increase in ear thickness
Recovered
Uninfected
4
24
48
72
Time after testing (hours)

in controls. The increase in ear thickness at 48 hours was significantly higher in the infected group ($p < 0.05$), but the increase at 24 hours was not found to be significant.

DISCUSSION

These experiments show that whereas acute infections such as P. b. berghei in the CBA mouse and P. b. yoelii in the T.O. mouse severely impair the ability to respond to the skin sensitizing agent oxazolone, the comparatively mild self limiting infection P. b. yoelii in the CBA mouse does not produce any detectable decrease in responsiveness to the same antigen. It was demonstrated previously that on day 11 of a P. b. yoelii infection in CBA mice there was a significant reduction in the proportion of PHA responsive cells in the spleen. Despite this, the ability of P. b. yoelii infected mice to elicit a cell mediated response to oxazolone, appears to be unimpaired. A similar observation was made by Greenwood (1971) who found that Balb/C mice infected with P. b. yoelii responded normally to oxazolone. He also demonstrated that graft rejection was not affected by these infections.

The mice in the present series of experiments were sensitized on day 10 of a P. b. yoelii infection, a time at which a great deal of T cell mitotic activity was shown to occur. It was suggested that the decreased responsiveness to PHA and thymus dependent antigens such as SRBC might in fact be related to such activity, in that a large part of the T cell pool was "preoccupied"

responding to the parasite and hence was probably unable to react effectively to a second antigen. However, such "pre-emption" of the T cell pool, if it does occur, does not seem to affect the response to oxazolone and hence may be operative only against certain antigens. T cell mitotic activity was found to decline rapidly to control levels around day 14 to 15. It would be interesting to determine whether this pattern of mitotic activity was altered in mice sensitized to oxazolone - a potent stimulator of T cell mitosis (Davies et al., 1969b).

It would also be useful to study the response to oxazolone employing smaller amounts of the antigen for sensitization in view of the observation of Ptak et al. (1970). These workers found that CBA mice with murine leprosy did not respond significantly to oxazolone when sensitized with 0.05 ml. If larger sensitizing doses (0.1 ml) were used sensitivity developed.

P. b. berghei infected CBA mice were unable to respond significantly to oxazolone. This infection was also shown in the present investigation to cause a marked depletion of the splenic PHA responsive population. Whitmore (1973) has shown that delayed hypersensitivity responses to sheep red blood cells (SRBC) and methylated human serum albumin (MHSA) are depressed in T.O. mice infected with P. b. berghei. Jerusalem et al. reported that mice infected with the K 173 strain of P. b. berghei failed to reject homogenic and allogenic skin grafts. (Jerusalem, cited by Salaman et al., 1969; Sengers, Jerusalem and Doesburg, 1971).

It would seem that the immune defect in P. b. berghei infected mice is generalised and results in a state of reduced responsiveness to most antigens. The chromosome marker experiments described previously suggest that the immunological defect probably stems from the inability of the T cell pool to respond effectively to the parasite. This deficiency in the functional capacity of the T cell pool probably extends to other antigens as well. As was discussed previously, the infections induce high plasma corticosterone levels and marked thymic cortical atrophy, and these factors probably contribute to the depressed response to oxazolone particularly during the later stages of the infection.

Steroids could exert their effects directly on the T cell. On the other hand, it has been suggested that they could exert their immunosuppressive effects by acting at the site of application of the oxazolone so as to prevent the release of antigenic fragments from epidermal cells, or by impairing movement of polymorphs and monocytes to the skin site, thus preventing induction of contact sensitivity. The possibility that such mechanisms might be important in the steroid induced suppression of contact sensitivity to oxazolone has been discussed by de Sousa and Fachet (1972).

T.O. mice infected with P. b. yoelii provided an intermediate situation between the two models discussed so far. During an infection they show considerably decreased responsiveness to oxazolone. The high steroid levels and thymic cortical involution demonstrated in these infections probably contributes to the

depressed responsiveness observed. About 60% of these mice eliminate the infection and show a rapid return to normality. The enhanced response to oxazolone elicited in these mice on day 29 at 24 and 48 hours correlates well with an observation made earlier in this investigation, that such recovered T.O. mice had significantly increased thymus weights - which might be indicative of an expansion of the T cell pool in these recovered mice.

Table 4.3 Development of contact sensitivity in CBA mice infected with P. b. yoelii

Group	Mean percentage increase in ear thickness ± S.E.			
	Time (hrs) after testing			
	4	24	48	72
CBA mice uninfected	36.00 ±1.69	74.58 ±4.76	64.50 ±3.20	53.33 ±6.70
CBA mice P. b. y. infected	30.00 ±0.82	70.88 ±4.28	62.50 ±6.84	42.73 ±7.70
significance	$p < 0.05$	NS	NS	NS

Table 4.4 Development of contact sensitivity in CBA mice infected with P. b. berghei

Group	Mean percentage increase in ear thickness ± S.E.			
	Time (hrs) after testing			
	4	24	48	72
CBA mice uninfected	30.00 ±1.50	82.50 ±10.30	65.00 ±5.00	57.50 ±2.50
CBA mice P. b. b. infected	27.66 ±0.84	29.00 ±3.60	20.33 ±3.40	12.50 ±7.50
significance	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$

Table 4.5 Development of contact sensitivity in T.O. mice infected with P. b. yoelii

Group	Mean percentage increase in ear thickness ± S.E.		
	Time (hrs) after testing		
	4	24	48
T.O. mice uninfected	77.17 ±20.06	97.75 ±9.01	54.46 ±11.70
T.O. mice P. b. y. infected	19.62 ±5.15	38.70 ±3.68	29.25 ±2.24
significance	$p < 0.01$	$p < 0.001$	$p < 0.05$

Table 4.6 Development of contact sensitivity in T.O. mice recovered from a P. b. yoelii infection.

Group	Mean percentage increase in ear thickness ± S.E.		
	Time (hrs) after testing		
	4	24	48
T.O. mice uninfected	60.90 ±6.52	99.12 ±9.56	55.50 ±7.85
T.O. mice recovered from a P.b.y. infection	56.75 ±8.84	122.25 ±18.17	91.50 ±10.59
significance	NS	NS	$p < 0.05$

CHAPTER 5

THE PASSIVE TRANSFER OF IMMUNITY WITH SERUM AND CELLS

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INTRODUCTION

In earlier sections of this thesis, the nature of the host response to the murine malarial parasite P. b. yoelii was investigated using techniques of in vivo lymphocyte depletion and reconstitution. The interaction between host and parasite was also characterized using mice carrying chromosomally distinguishable cell populations. The present section is concerned with the use of serum and cell transfers from immune to non immune mice, to analyse further the host response in malaria.

Such serum and cell transfers offer a direct method by which to evaluate humoral and cellular contributions towards eliciting and maintaining a state of effective immunity in these infections. It provides a particularly powerful analytical tool since it allows manipulation (a) of the donor, (b) of the cells or serum while in transit between donor and recipient, and (c) of the recipients themselves.

In the present investigation, use has been made in particular, of the T-cell deprived recipient. It was shown that such mice lacked the endogenous apparatus necessary for protecting themselves against a P. b. yoelii infection. However, it is known that T cell deprivation suppresses both cell mediated immunity (CMI) and humoral antibody responses to thymus dependent antigens. To define the nature of the immunological lesion in these animals more precisely, selective restoration of responsiveness was attempted with serum and cells.

MATERIALS AND METHODS

1. ANIMALS

Male CBA mice (aged 7 to 15 weeks) were used in these experiments. Hyperimmune serum was raised in outbred T.O. Swiss mice aged 12 to 15 weeks.

2. T CELL DEPRIVATION

CBA mice were thymectomized, X-irradiated and reconstituted with syngeneic bone marrow - as described in Chapter 2.

3. THE PARASITE

The parasite used in these experiments was P. berghei yoelii. Storage of parasite and preparation of infective inocula were carried out as described previously.

4. PREPARATION OF ANTISERA

4.1 Immune serum

CBA mice were infected with 1×10^5 P. b. yoelii parasitized erythrocytes. Two weeks after the parasites had been eliminated from the peripheral blood the mice were bled from the retro-orbital sinus. The blood was allowed to clot at room temperature, left overnight at $4^{\circ}C$ and the serum separated by centrifugation at 1,000g for 10 min. The serum was inactivated at $56^{\circ}C$ for 30 min and stored at $-70^{\circ}C$.

4.2 Hyperimmune serum

Outbred T.O. mice were infected with 1×10^5 P. b. yoelii parasitized erythrocytes. Mice which recovered from the primary infection were challenged with an inoculum of 1×10^6 parasitized erythrocytes at weekly intervals for 6 weeks. 1 week after the last inoculum the mice were bled from the retro-orbital sinus and the serum was separated, inactivated and stored as before.

5. PREPARATION OF SPLEEN CELL SUSPENSIONS

Donor mice were killed by cervical dislocation. The spleens were removed, cut into small fragments and passed through fine stainless steel sieves into cold 199 medium (Wellcome). The cell suspension was aspirated through a 25G needle, washed twice in 199 medium, counted in a haemocytometer, and adjusted to the required cell concentration.

6. PREPARATION OF AKR ANTI-THETA/C_3H SERUM

The anti theta/C3H serum used in these experiments was a gift from Dr R.S. Kerbel of the Chester Beatty Research Institute, London. It was prepared in the following manner according to the method of Reif and Allen (1966). Three month old female AKR mice were injected with 20×10^6 washed thymocytes at weekly intervals, except that an interval of 6 weeks separated the second and third injections. The mice were bled 9 days after the seventh and final injection, the serum was pooled, inactivated at $56^{\circ}C$ for 30 min, and stored neat, in small aliquots, at $-20^{\circ}C$. This antiserum

killed 30 to 35% of CBA spleen cells at dilutions up to 1 : 16.

7. TREATMENT OF CELLS WITH ANTI-THETA ANTISERUM

Spleens from recovered mice were processed in 199 medium as described above to give a cell suspension, the concentration of which was adjusted to 20×10^6 cells/ml. 1.5 ml aliquots of this cell suspension (i.e. $2\text{-}3 \times 10^7$ cells) were transferred to polystyrene tubes and centrifuged at 250g for 7 min. The supernatant was decanted and the cells were resuspended in 1 ml of anti-theta/C3H antiserum or normal AKR serum diluted 1 : 10 with 199 medium. The cells were incubated at 37°C for 20 min. The cells were then sedimented at 250g for 5min, all anti-theta antiserum was removed and the cells resuspended in 1 ml of fresh guinea pig serum diluted 1 : 8 with 199 medium. The cells were now reincubated at 37°C for a further 30 min. Finally, the cells were resedimented, washed once in medium 199 and diluted to the required cell concentration. Cell viability was determined by the trypan blue exclusion test as described by Boyse et al. (1968).

8. TREATMENT OF CELL DONORS WITH CYCLOPHOSPHAMIDE

Cell donors were treated with a single dose of cyclophosphamide (Endoxana) 300 mg/Kg body weight. Spleen cells were harvested from these mice 3 days after treatment. Some spleens from treated mice were fixed in Carnoy's fluid, processed according to standrad histological methods, and were stained in methyl green pyronin or haematoxylin and eosin.

9. ANALYSIS AND PRESENTATION OF DATA

All experimental groups consisted of at least 5 mice. Blood parasitaemias were determined on individual mice as described in Chapter 1. Mean percentage parasitaemias and standard errors were calculated. Where necessary, Student's t-test was used to determine the significance of differences between groups. The results of these experiments are presented in the form of graphs.

EXPERIMENTS AND RESULTS

P. b. yoelii infections in CBA mice: A note on the pattern of infection

Infection of intact CBA mice with 1×10^4 P. b. yoelii parasitized erythrocytes resulted in a very characteristic pattern of infection. The parasite could be detected in the peripheral blood 2 to 4 days after infection; the parasitaemia increased steadily and peaked around day 10 to 12 after which the parasites were eliminated from the peripheral blood fairly rapidly. The peripheral blood was usually aparasitaemic 16 to 18 days after infection.

In the present series of experiments it was found that although all infections in intact untreated CBA mice conformed to this basic pattern, there was a certain amount of variation in the peak parasitaemias recorded in different experiments. This is thought to be due to an age factor which could not be rigidly controlled. Younger mice aged 7 to 8 weeks tended to show peak infections of 5 to 6%, while in older mice (12 to 15 weeks) the peak

parasitaemias were 1 to 2%. For practical reasons it was not possible to use mice of the same age for the entire series of experiments described, but all mice within each experiment were age matched.

1. TRANSFER OF IMMUNITY WITH SERUM

Attempts were made to transfer immunity to non immune hosts by the passive administration of serum. The effects of two pools of sera were studied.

1. Serum from CBA mice 15 days after recovery from a primary infection. This serum was designated immune serum (IMS).
2. Serum from outbred T.O. mice which had been repeatedly challenged, after recovery from a primary infection. This was termed hyper-immune serum (HPS).

All sera were administered intravenously into the lateral tail vein and mice were challenged 1 to $1\frac{1}{2}$ hours later with the parasite inoculum, intraperitoneally.

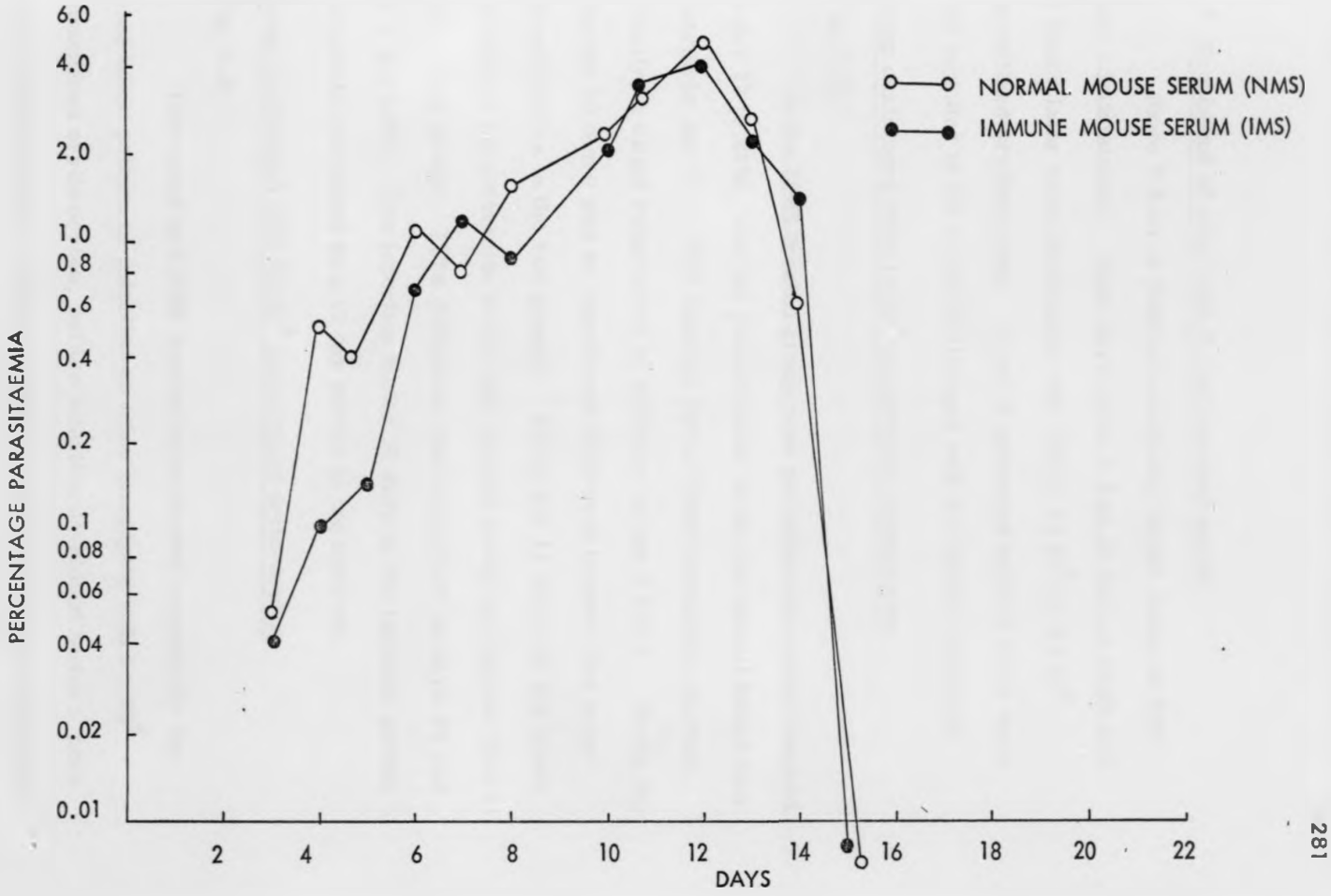
The effect of immune serum (IMS)

1.1 Treatment of mice with 0.2 ml immune serum

(Fig. 5.1)

Intact CBA mice were given 0.2 ml of IMS or NMS (normal mouse serum), and were infected with 1×10^4 *P. b. yoelii* parasitized erythrocytes $1\frac{1}{2}$ hours later. Mice treated with IMS showed no significant difference in parasitaemia from the NMS treated group.

Fig. 5.1 Mean percentage parasitaemias in normal CBA mice treated with 0.2 ml of (IMS) immune mouse serum or (NMS) normal mouse serum and challenged with 1×10^4 *P.b. yoelii* parasitized RBC.


NORMAL MOUSE SERUM (NMS)
IMMUNE MOUSE SERUM (IMS)
PERCENTAGE PARASITAEMIA
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
22
DAYS

1.2 Treatment of mice with 0.5 ml immune serum

Since 0.2 ml of IMS had no effect, larger doses of IMS were administered. Mice were given 0.5 ml of IMS or NMS and $1\frac{1}{2}$ hours later were challenged with either 1×10^4 or 5×10^4 parasitized erythrocytes. A set of untreated control mice were also included in the group challenged with the larger inoculum.

Group challenged with 1×10^4 parasitized erythrocytes

(Fig. 5.2)

In the NMS treated group peak parasitaemias were reached on day 12 (3.21%), and the parasitaemia in the peripheral blood was cleared by day 17. The immune serum (IMS) recipients showed, initially, a slight retardation of infection on day 3 and 4. From day 5 to day 10 there was no significant difference between the mean parasitaemias in the two groups. From day 11 onwards the mean percentage parasitaemia in the IMS treated group was higher than in the control group. (The difference was significant on days 14 and 15 - $p < 0.05$.) The infection lasted 19 days in the immune serum recipients compared to a 17 day period in the controls.

Group challenged with 5×10^4 parasitized erythrocytes

(Fig. 5.3)

Untreated and NMS treated mice showed essentially the same basic pattern of infection as mice inoculated with 1×10^4 parasitized erythrocytes, but the infection developed faster; peak parasitaemias were recorded on day 12 and 11 in the untreated and

Fig. 5.2 Mean percentage parasitaemias in normal CBA mice treated with 0.5 ml (IMS) immune mouse serum or (NMS) normal mouse serum and challenged with 1×10^4 P.b. yoelii parasitized RBC.

10.0

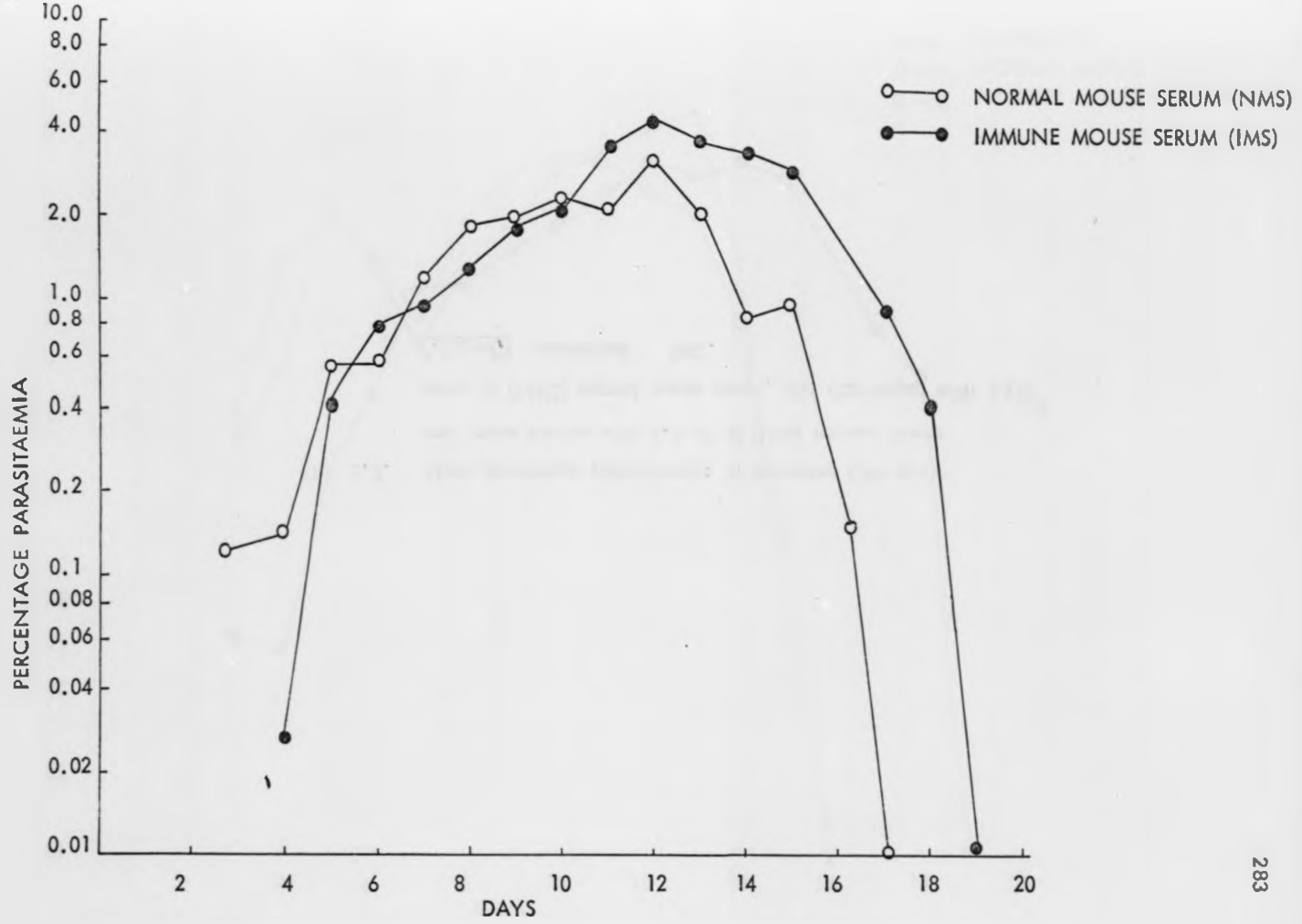
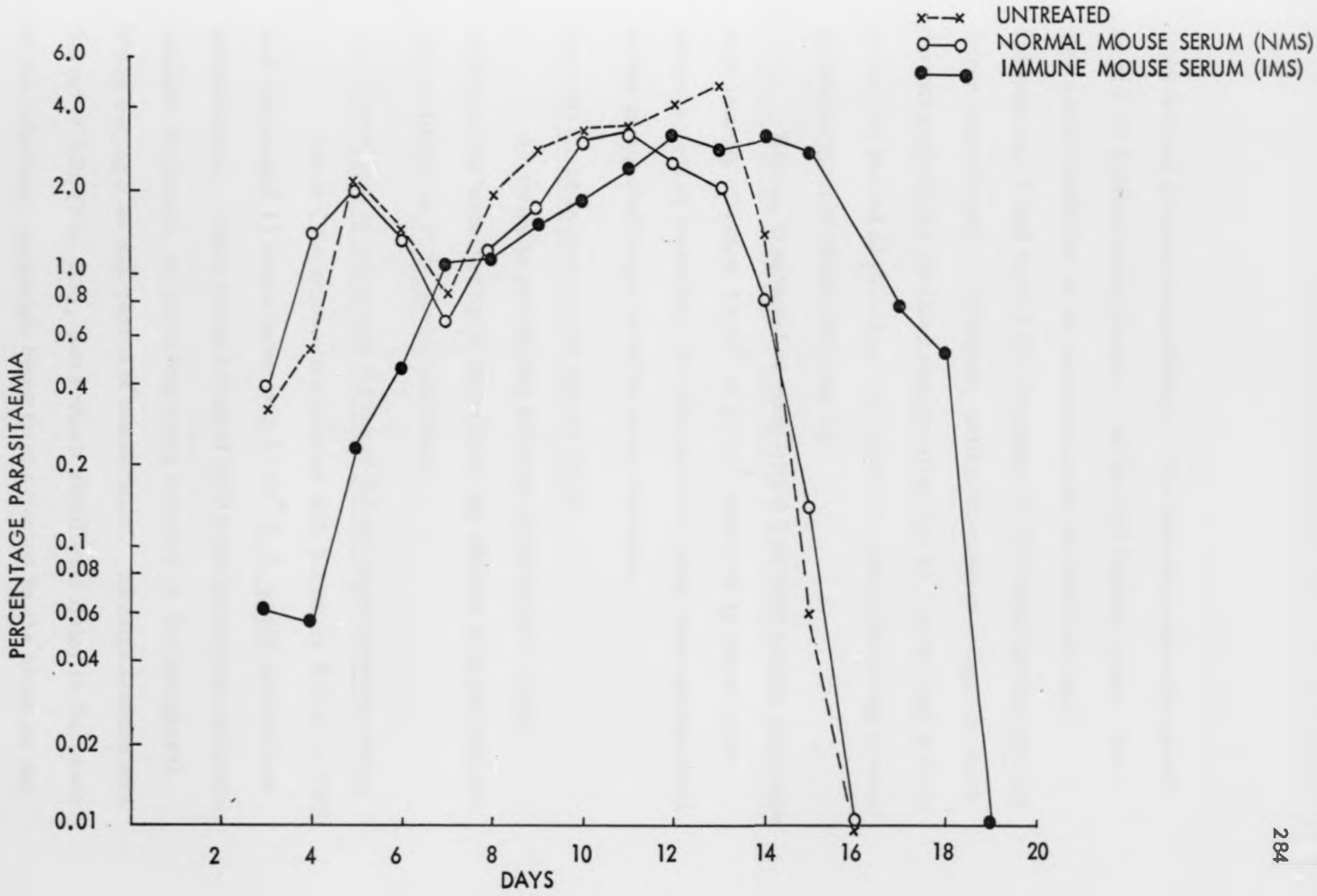

NORMAL MOUSE SERUM (NMS)
IMMUNE MOUSE SERUM (IMS)
PERCENTAGE PARASITAEMIA
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
DAYS

Fig 5.3. Mean percentage parasitaemias in untreated CBA mice and mice treated with 0.5 ml of (IMS) immune mouse serum or (NMS) normal mouse serum, and challenged with 5×10^4 P. b. yoelii parasitised RBC.


UNTREATED
NORMAL MOUSE SERUM (NMS)
IMMUNE MOUSE SERUM (IMS)
PERCENTAGE PARASITAEMIA
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
DAYS

NMS treated groups respectively. The infection was eliminated by day 16 in both these groups. In the IMS treated group, there was some inhibition of parasitaemia over the first six days; between day 7 and day 13 the infections in the three groups did not differ significantly. However, unlike the control groups in which the parasitaemias declined rapidly after day 13, in the IMS treated group the period of infection was extended, parasites being present in the peripheral blood until day 19.

Hence, treatment of mice with 0.5 ml IMS before challenge with inocula of either 1×10^4 or 5×10^4 appeared to cause some enhancement of infection, the enhancement being more pronounced in the group challenged with the large inoculum.

The effects of hyperimmune serum (HPS)

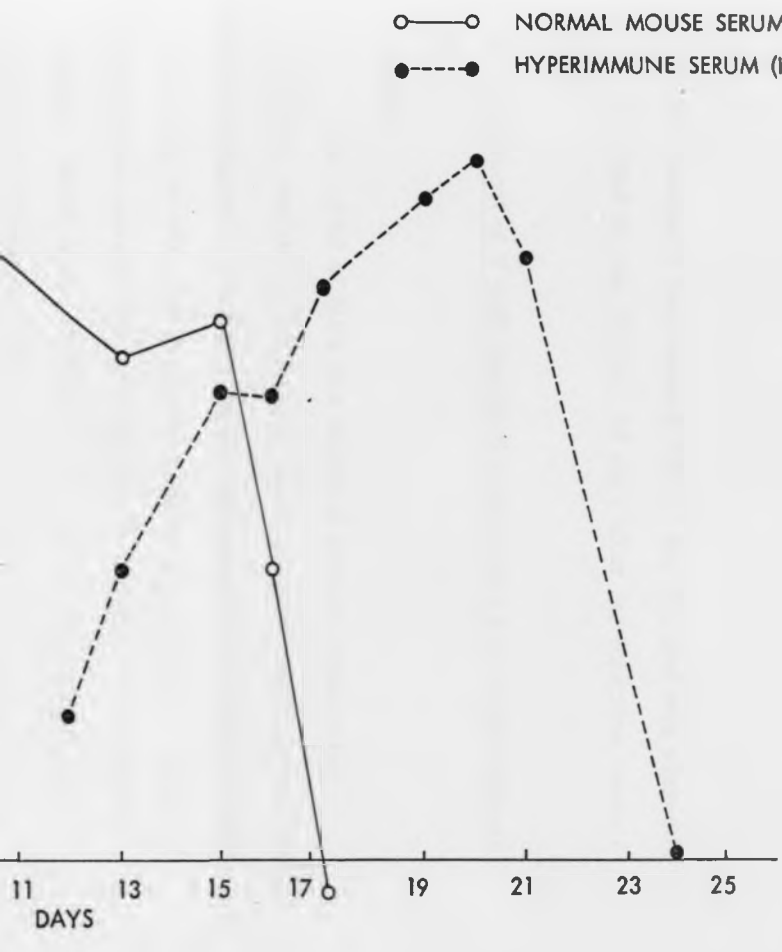
As immune serum had little or no protective effect, experiments were set up to investigate the effects of hyperimmune serum (HPS) on P. b. yoelii infections.

1.3 Treatment of mice with 0.2 ml or 0.5 ml hyperimmune serum

Intact CBA mice were treated with 0.2 ml or 0.5 ml of HPS and challenged $1\frac{1}{2}$ hours later with 1×10^4 P. b. yoelii parasitized erythrocytes. Mice given 0.5 ml of HPS were protected completely against challenge, no parasites being detected in the peripheral blood during a 20 day period of observation. In mice treated with 0.2 ml of HPS (Fig. 5.4), there was a significant delay in the onset of the infection, parasites being first detected in the blood on day 12 (cf. day 3 in controls). Thereafter the parasitaemia increased

Fig. 5.4 Mean percentage parasitaemias in normal mice treated with 0.2 ml (HPS) hyperimmune serum or (NMS) normal mouse serum, and challenged with 5×10^4 P.b. yoelii parasitized RBC.

PERCENTAGE PARASITAEMIA
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
3
5
7
9


NORMAL MOUSE SERUM (NMS)
HYPERIMMUNE SERUM (HPS)
11
13
15
17
19
21
23
25
DAYS

steadily, reached a maximum 2.02% on day 20, and was cleared from the blood by day 25 i.e., 13 days after the infection became patent.

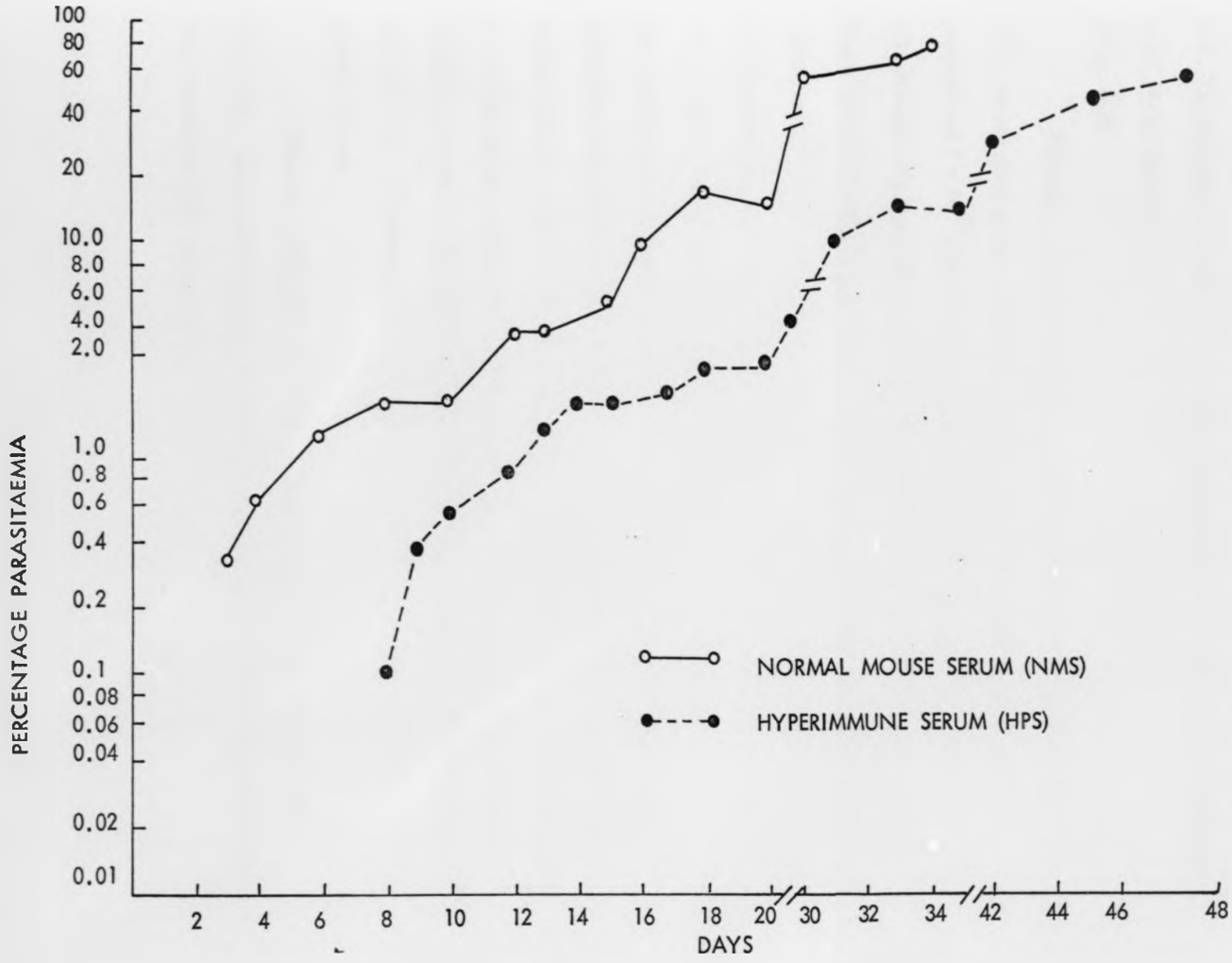
1.4 Treatment of T cell deprived mice with 0.5 ml hyperimmune serum

(Fig. 5.5)

As 0.5 ml of HPS was shown to protect intact mice completely, the ability of this serum to protect "deprived" recipients was investigated. Such recipients had been shown to be incapable of mounting an effective response against P. b. yoelii. 0.5 ml of hyperimmune serum was administered to normal and "deprived" recipients which were challenged with 5×10^4 P. b. yoelii parasitized RBC ($1\frac{1}{2}$ hours later).

As in the previous experiment, 0.5 ml HPS conferred complete protection, in this case against a larger challenge inoculum (5×10^4 P. b. yoelii). In T-cell deprived mice, however, the serum produced only a transient inhibitory effect on parasitaemia. The parasitaemia became patent in the HPS treated group on day 8 compared with day 3 in the NMS treated controls. Thereafter the parasitaemia increased steadily and the infection proved fatal in all HPS treated "deprived" mice. The mean percentage parasitaemia in the HPS treated group was lower than in the controls throughout most of the infection, and no evidence of enhancement was seen in these serum treated "deprived" mice. The mean survival time of the hyperimmune serum recipients was 47 days compared to 38

Fig. 5.5 Mean percentage parasitaemias in T cell deprived mice treated with 0.5 ml (HPS) hyperimmune serum or (NMS) normal mouse serum, and challenged with 5×10^4 P.b. yoelii parasitized RBC.


PERCENTAGE PARASITAEMIA
100
80
60
40
20
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
20
30
32
34
42
44
46
48
DAYS
NORMAL MOUSE SERUM (NMS)
HYPERIMMUNE SERUM (HPS)

days in the NMS treated group.

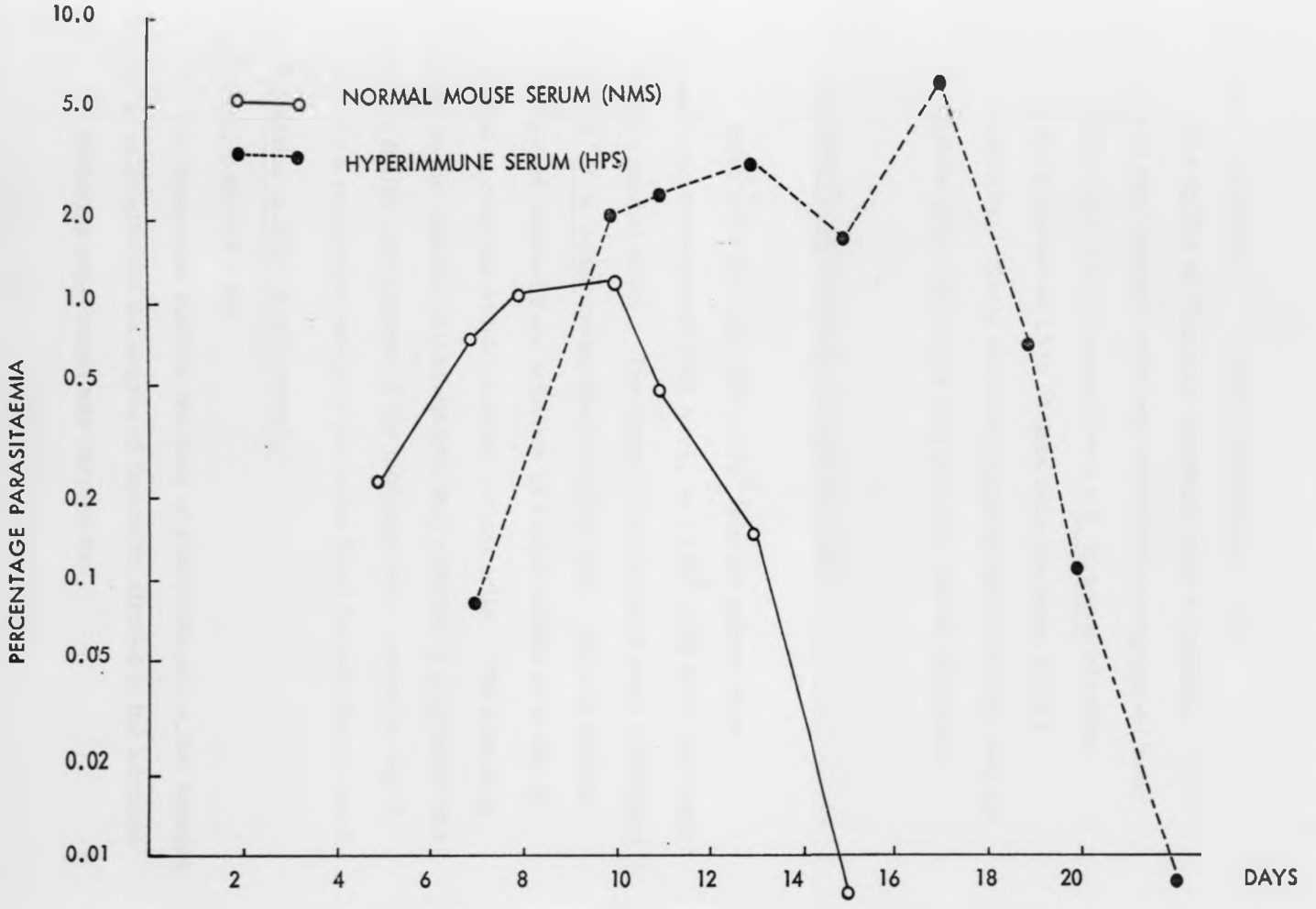
1.5 Treatment of mice with 0.5 ml hyperimmune serum and challenge with large inocula

(Fig. 5.6)

Finally, an attempt was made to determine whether the HPS which had protected intact mice completely against parasite inocula of 1×10^4 and 5×10^4 P. b. yoelii could protect mice as effectively against still larger challenge inocula. Intact CBA mice were treated with 0.5 ml HPS or NMS and challenged $1\frac{1}{2}$ hours later with 1×10^5 parasitized erythrocytes. All hyperimmune serum (HPS) recipients became infected. The parasitaemia was patent on day 7 i.e. 4 days later than in the NMS treated group. The mean percentage parasitaemia in the HPS treated group throughout the infection was higher than in the control group at a comparable stage of infection. Peak parasitaemia in the HPS group was 6.0% compared to 1.23% in the controls and was recorded 10 days after the infection became patent. The HPS treated group eliminated the infection by day 23, i.e. 16 days after the appearance of parasites in the peripheral blood.

Hence, although the HPS initially delayed the onset of the infection, once the parasitaemia became patent the resulting infection was considerably enhanced.

Fig. 5.6 Mean percentage parasitaemias in normal CBA mice treated with 0.5 ml (HPS) hyperimmune serum or (NMS) normal mouse serum, and challenged with 1×10^5 *P.b. yoelii* parasitized RBC.


10.0
5.0
2.0
1.0
0.5
0.2
0.1
0.05
0.02
0.01
PERCENTAGE PARASITAEMIA
NORMAL MOUSE SERUM (NMS)
HYPERIMMUNE SERUM (HPS)
2
4
6
8
10
12
14
16
18
20
DAYS

2. THE TRANSFER OF IMMUNITY WITH CELLS

The ability of "immune" lymphoid cells to transfer immunity to non-immune hosts was investigated using spleen cells from donors which had recovered from a P. b. yoelii infection. All cells were harvested 15 to 20 days after recovery from a primary infection. Cells were administered intravenously, and the cell recipients were challenged with parasite inocula intraperitoneally.

2.1 The transfer of immunity with spleen cells

(Fig. 5.7)

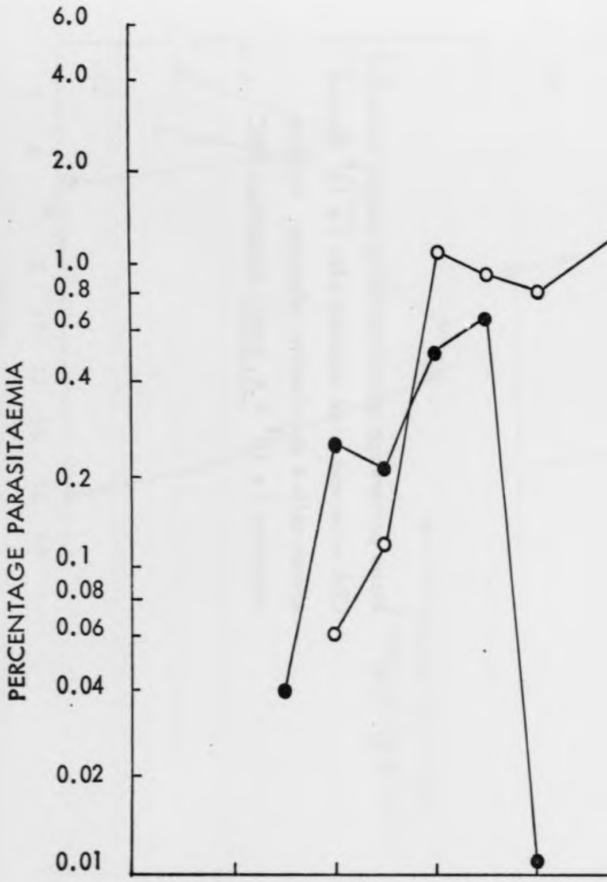
Mice were injected with 1×10^8 immune spleen cells obtained from "recovered" CBA mice, or 1×10^8 cells from untreated CBA mice (normal cells). Two hours later the mice were challenged with 1×10^4 P. b. yoelii parasitized erythrocytes. Immune spleen cell recipients cleared their infection in 8 days compared to the 15 day period in controls which received normal cells. The course of infection in the immune cell recipients was modified in that there was initially a faster development of the infection which peaked on day 7, followed by a rapid elimination of parasites from the peripheral blood.

2.2 Variation in time of cell transfer

(Fig. 5.8a, 5.8b and 5.8c)

To determine whether the time of administration of the immune lymphoid cells affected the degree of immunity elicited in the adoptive host, the following experiment was carried out.

Fig. 5.7 Mean percentage parasitaemias in normal CBA mice injected with 1×10^8 immune spleen cells or normal spleen cells, and challenged with 1×10^4 P b. yoelii parasitized RBC.


PERCENTAGE PARASITAEMIA
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8

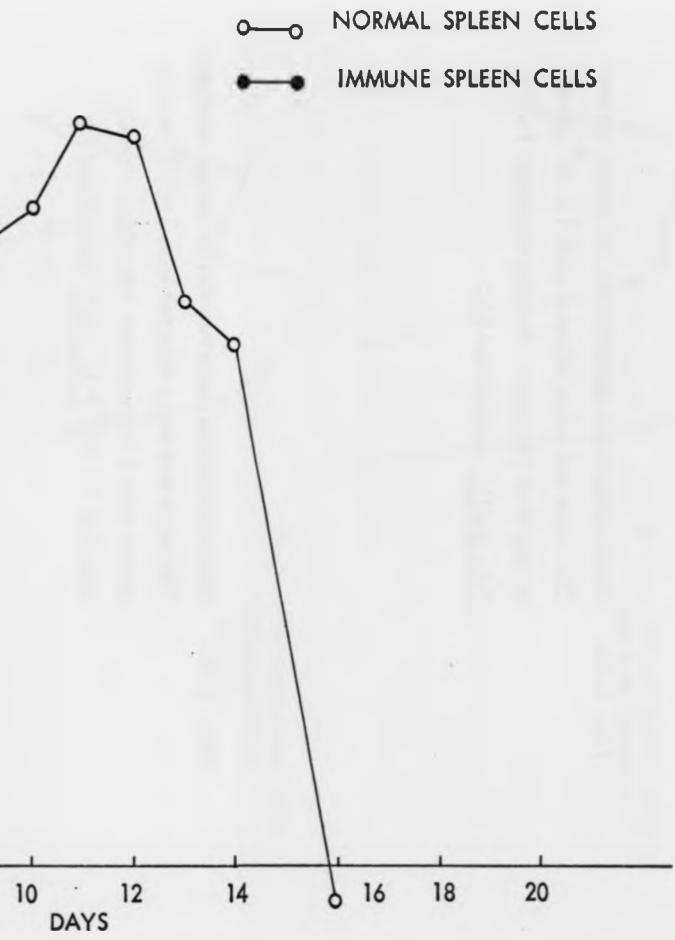
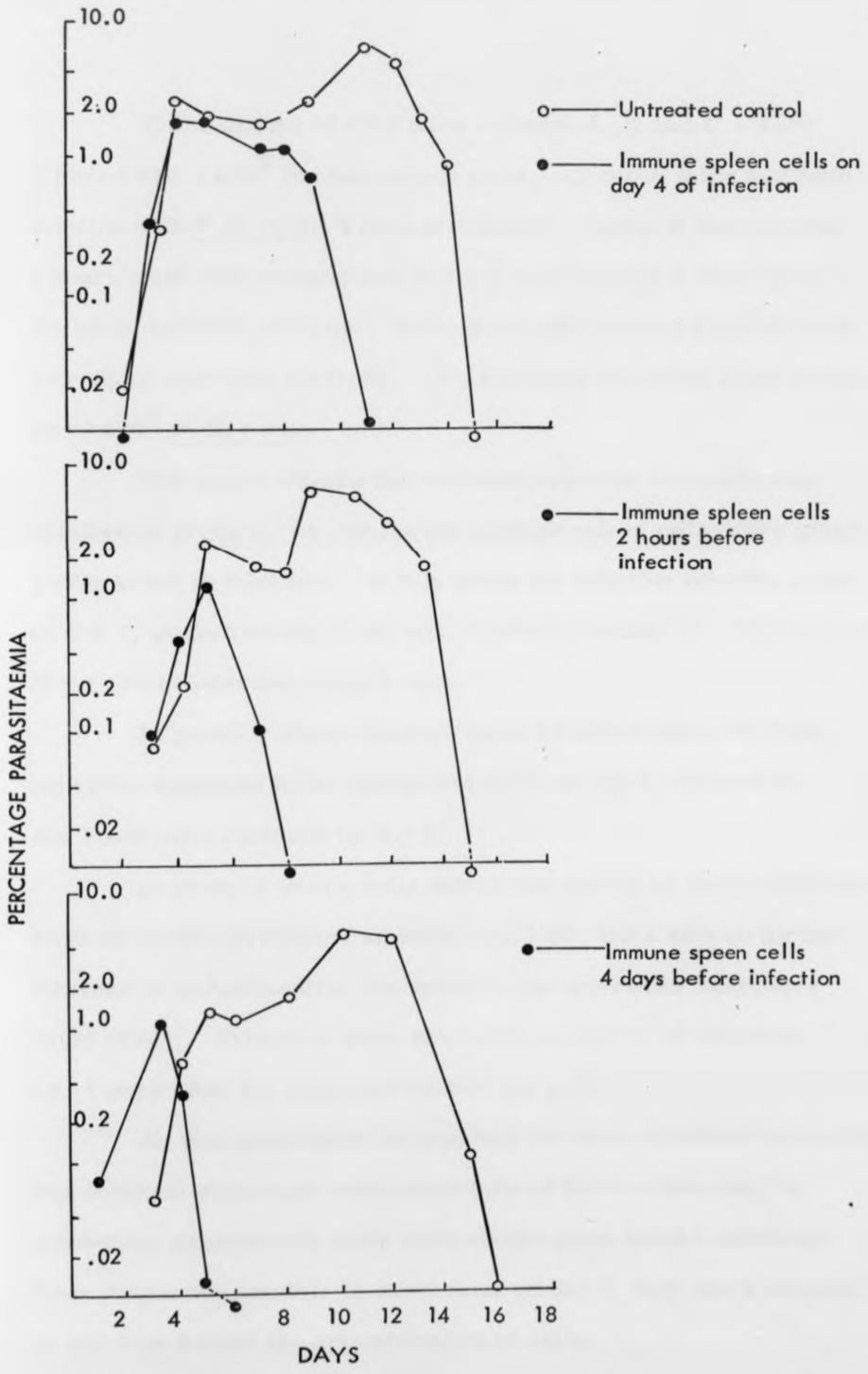

NORMAL SPLEEN CELLS
IMMUNE SPLEEN CELLS
10
12
14
16
18
20
DAYS

Fig. 5.8a Mean percentage parasitaemias in normal untreated CBA mice and mice injected with 1×10^8 spleen cells on day 4 of infection. Parasite inoculum 1×10^4 *P.b. yoelii* parasitized RBC.

Fig. 5.8b Mean percentage parasitaemias in normal untreated CBA mice and mice injected with 1×10^8 immune spleen cells 2 hours before infection. Parasite inoculum 1×10^4 *P.b. yoelii* parasitized RBC .

Fig. 5.8c Mean percentage parasitaemias in normal untreated CBA mice and mice injected with 1×10^8 immune spleen cells 4 days before infection. Parasite inoculum 1×10^4 *P.b. yoelii* parasitized RBC.


10.0
2.0
1.0
0.2
0.1
.02
Untreated control
Immune spleen cells on day 4 of infection
Immune spleen cells 2 hours before infection
Immune speen cells 4 days before infection
PERCENTAGE PARASITAEMIA
2
4
8
10
12
14
16
18
DAYS

Three groups of CBA mice - groups A, B and C - were injected with 1×10^8 immune spleen cells. Group A mice had been infected with P. b. yoelii 4 days previously. Group B was infected 2 hours after cell transfer and group C was infected 4 days after the administration of cells. Each group was infected together with a group of untreated controls. The challenge inoculum in all cases was 1×10^4 P. b. yoelii.

The result showed that the most effective immunity was obtained in group C, i.e. where the immune spleen cells were given 4 days prior to infection. In this group the infection became patent on day 1, peaked on day 3 and was eliminated on day 5. The duration of the patent infection being 4 days.

In group B which received cells 2 hours before infection, parasites appeared in the peripheral blood on day 3, peaked on day 5 and were removed by day 8.

In group A where cells were given during an active infection when the mean percentage infection was 1.80, there was no further increase in parasitaemia; the spleen cells apparently exerting a rapid effect. Parasites were eliminated on day 11 of infection, i.e. 7 days after the administration of the cells.

As this experiment showed that the most effective immunity was obtained when cells were administered before infection, in subsequent experiments cells were always given before challenge. When it was not possible to infect mice on day 4, they were infected on day 5 or 6 after the administration of cells.

2.3 Dose response relationship

(Fig. 5.9)

In the experiments described so far, cell recipients were inoculated with 1×10^8 spleen cells - this being equivalent to a donor recipient ratio of 1 : 1. The present experiment was carried out to determine the effects of variation in cell numbers on the degree of immunity obtained in the adoptive host.

Doses of immune spleen cells ranging from 1×10^6 to 2×10^8 were given to groups of intact CBA mice. 6 days later these mice were challenged together with a group of untreated controls with an inoculum of 1×10^4 P. b. yoelii parasitized erythrocytes. Fig. 5.9 shows the varying degrees of protection conferred with graded doses of immune spleen cells. The administration of large numbers of spleen cells (2×10^7 to 2×10^8) resulted in the elimination of the challenge organism within 7 to 8 days in contrast to its normal growth in untreated recipients. Smaller doses of immune cells (1×10^6) produced proportionally less protection, the infection persisting until day 10. The prolonged pre-patent period (4 days) in the groups receiving 1×10^8 and 2×10^8 cells, unlike in the previous experiment where recipients of 1×10^8 cells showed a rapid development of infection, might be related to the 6 day interval between cell transfer and parasite challenge used in the present experiment.

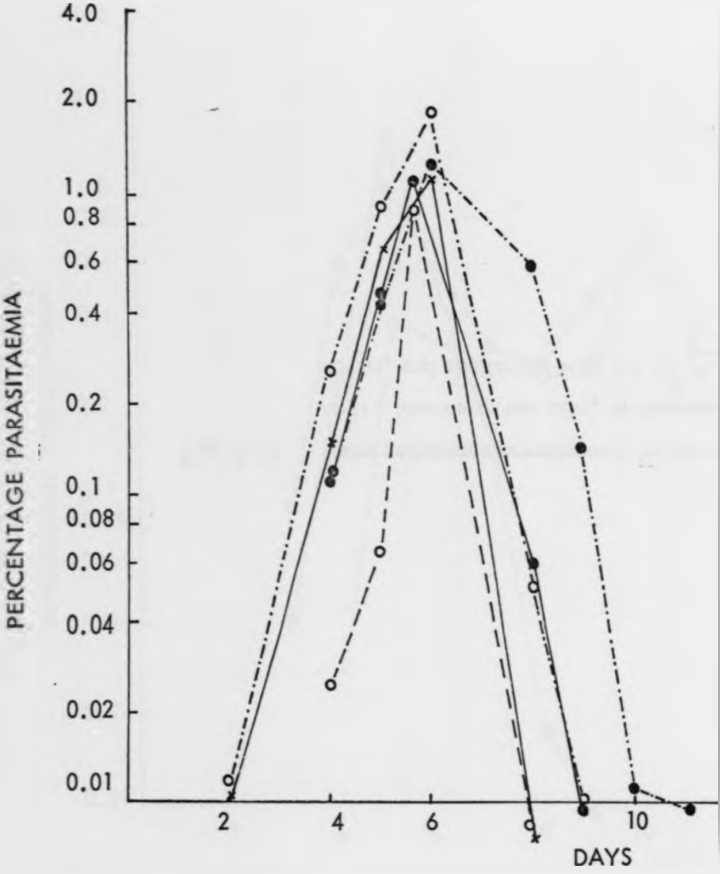
2.4 Treatment of cell donors with Cyclophosphamide

(Fig. 5.10)

The effect of Cyclophosphamide on the immune cells of the

Fig. 5.9

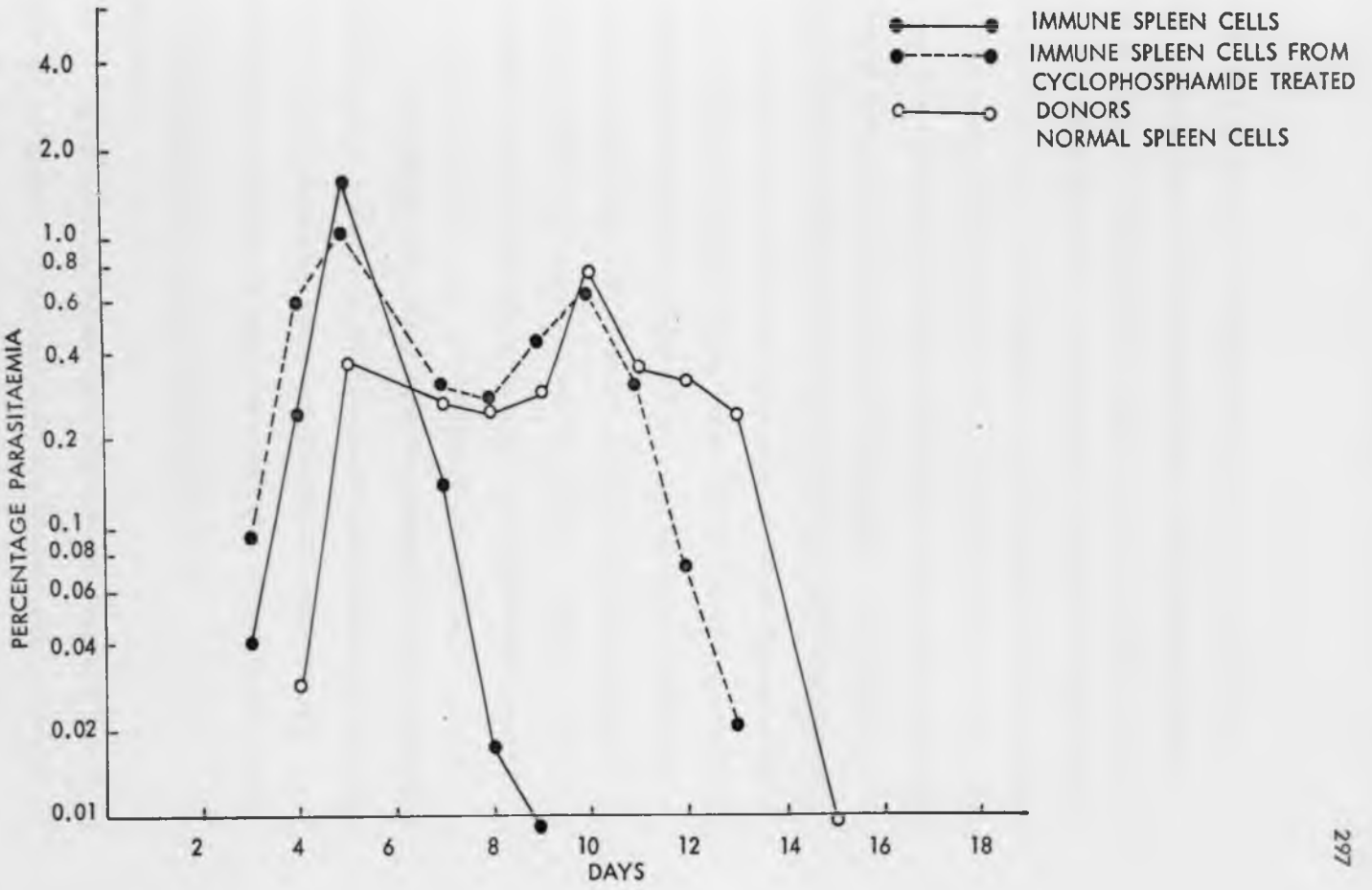
Mean percentage parasitaemias in normal CBA mice injected with varying numbers of immune spleen cells and challenged with 1×10^4 *P.b. yoelii* parasitized RBC. (The untreated control group is not shown on this graph.)


4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
PERCENTAGE PARASITAEMIA
2
4
6
10
DAYS

2×10^8
1×10^8
2×10^7
1×10^7
1×10^6
IMMUNE SPLEEN CELLS

12
14
16
18

Fig. 5.10 Mean percentage parasitaemias in normal CBA mice treated with 1×10^8 normal spleen cells , immune spleen cells, or immune spleen cells from Cyclophosphamide treated donors, and challenged with 1×10^4 P.b. yoelii parasitized RBC.


IMMUNE SPLEEN CELLS
IMMUNE SPLEEN CELLS FROM CYCLOPHOSPHAMIDE TREATED DONORS
NORMAL SPLEEN CELLS
PERCENTAGE PARASITAEMIA
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
2
4
6
8
10
12
14
16
18
DAYS

donor was studied by treating half of a group of recovered mice with a single dose of Cyclophosphamide - 300 mg/Kg body weight. The protective capacity of the lymphoid cells from these animals, obtained three days later, was compared with that of cells from the untreated donors. 1×10^8 spleen cells of each type were transferred into two groups of untreated CBA mice, which together with a control group which received a similar number of normal spleen cells were infected five days later with 1×10^4 P. b. yoelii. The results (Fig. 5.10) showed that while immune cells from untreated donors were effective in transferring resistance, an equal number of cells from Cyclophosphamide treated donors did not transfer a comparable level of immunity to the adoptive host - the infection in the latter group persisting until day 13 unlike in the untreated immune cell recipients which cleared the infection by day 9. Hence, prior treatment with Cyclophosphamide appears to significantly reduce the ability of cells to confer protection on non immune recipients.

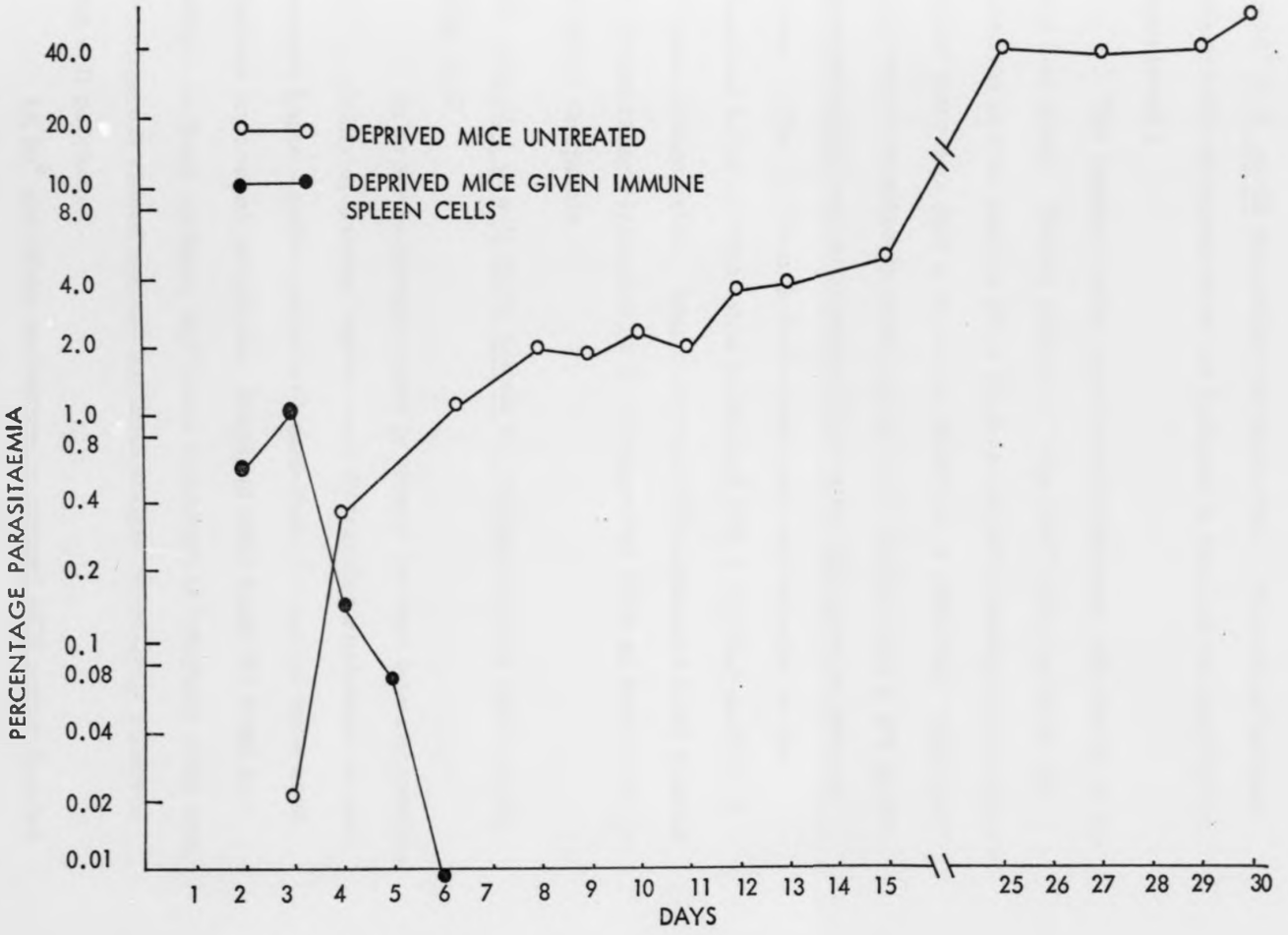
Histological examination of the spleens of Cyclophosphamide treated mice at the time of cell transfer showed that the lymphocytes in the 'thymus dependent' periarteriolar regions were largely unaffected, but there was extensive destruction of non T-cell areas.

2.5 Cell transfer into T-cell deprived recipients

(Fig. 5.11)

1×10^8 immune spleen cells were transferred into T-cell deprived recipients. Five days later the immune cell recipients and a group of untreated "deprived" mice were infected with

Fig. 5.11 Mean percentage parasitaemias in T cell deprived mice injected with 1×10^8 immune spleen cells and in untreated deprived mice challenged with 1×10^4 P.b. yoelii parasitized RBC.


40.0
20.0
10.0
8.0
4.0
2.0
1.0
0.8
0.4
0.2
0.1
0.08
0.04
0.02
0.01
PERCENTAGE PARASITAEMIA
DEPRIVED MICE UNTREATED
DEPRIVED MICE GIVEN IMMUNE SPLEEN CELLS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 25 26 27 28 29 30
DAYS

1×10^4 P. b. yoelii parasitized erythrocytes. (Groups of normal spleen cell recipients were not included in this and the subsequent experiment.)

The immune cells transferred resistance effectively to the deprived mice. Unlike untreated "deprived" mice in which the infection proved fatal in 35 to 40 days (or HPS treated deprived mice where there was only a transient inhibition of infection) "deprived" mice which received immune spleen cells showed only a low grade infection which was eliminated 6 days after the infection became patent. The infection in these mice was very similar to that observed in intact CBA mice inoculated with a similar number of immune spleen cells. Hence immune cells appeared to be capable of transferring resistance to T-cell deprived mice as effectively as to intact recipients.

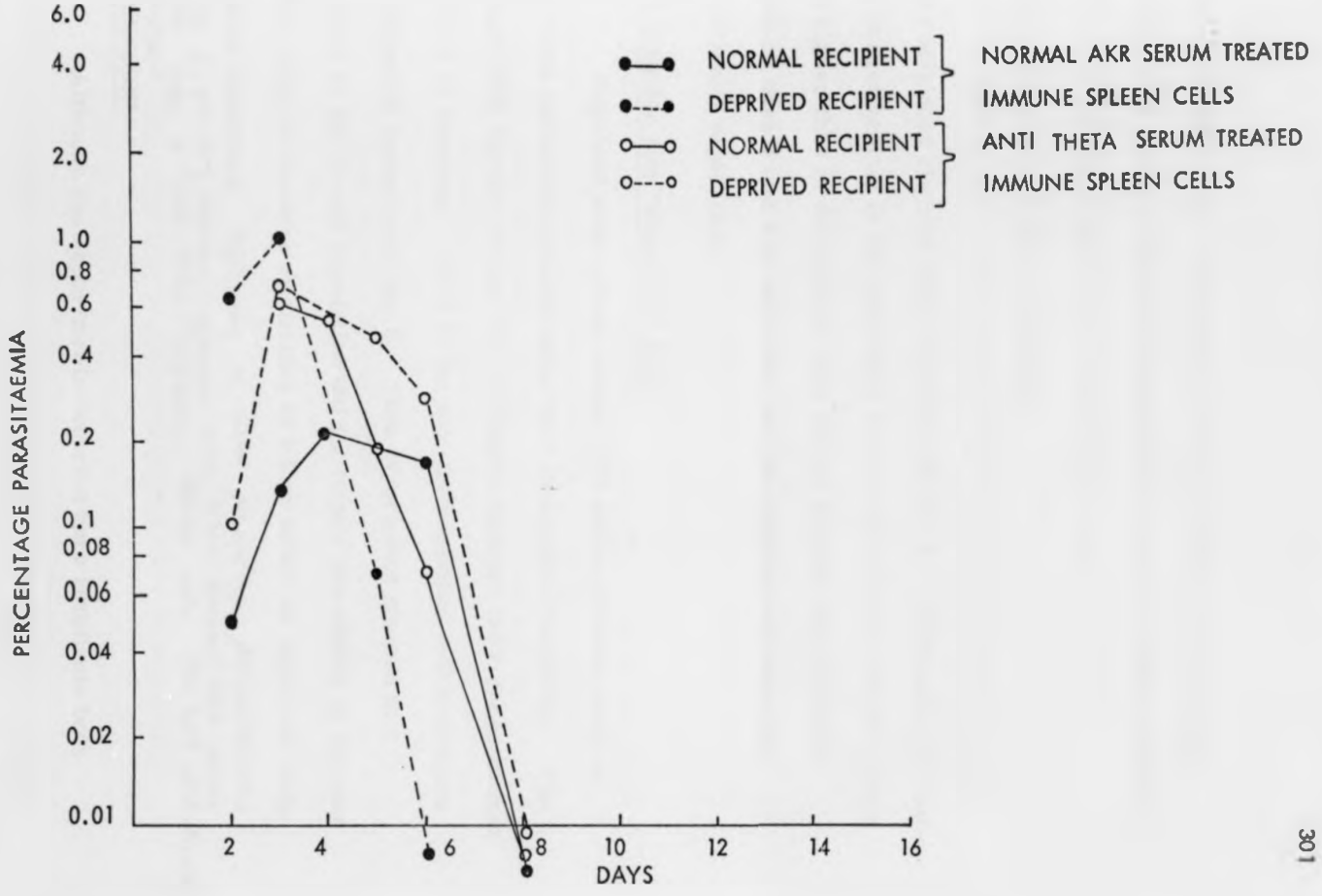
2.6 Transfer of anti-theta antiserum treated immune spleen cells (Fig. 5.12)

In order to identify more precisely the cell type responsible for the ability of immune spleen cells to transfer resistance to non-immune hosts - cells treated with anti-theta antiserum were used. Thymus processed peripheral lymphoid cells bear the theta iso-antigen on their surface, and hence treatment of lymphoid cells with an antiserum raised against the theta antigen selectively removes this cell population.

1×10^8 anti-theta antiserum or normal AKR serum treated immune spleen cells were injected into groups of intact and T-cell

Fig. 5.12

Mean percentage parasitaemias in normal and T cell deprived recipients injected with anti-theta antiserum treated immune spleen cells, or in normal and T cell deprived recipients injected with normal AKR serum treated immune spleen cells. Challenge inoculum 1×10^4 *P.b. yoelii* parasitized RBC.


6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
PERCENTAGE PARASITAEMIA
2
4
6
8
10
12
14
16
DAYS
NORMAL RECIPIENT
DEPRIVED RECIPIENT
NORMAL AKR SERUM TREATED
IMMUNE SPLEEN CELLS
NORMAL RECIPIENT
DEPRIVED RECIPIENT
ANTI THETA SERUM TREATED
IMMUNE SPLEEN CELLS

deprived CBA mice. 4 days later all cell recipients and groups of untreated control mice (both intact and "deprived") were infected with 1×10^4 P. b. yoelii parasitized erythrocytes.

Cell transfer into intact recipients

Both normal AKR serum and anti-theta antiserum treated cell recipients cleared their infections on day 8. The mean percentage parasitaemias in the anti-theta antiserum treated cell recipients was higher than in the normal AKR serum treated cell recipient group on days 3 and 4 of infection, but the differences were not statistically significant.

Cell transfer into "deprived" mice

Deprived mice given normal AKR serum treated cells or anti-theta antiserum treated cells were protected effectively. The normal AKR serum treated cell recipients cleared their parasitaemia on day 6 of infection - while in the anti-theta treated cell recipients the infection lasted until day 8. This experiment showed that removal of the T-cell population did not impair the ability of immune spleen cells to transfer resistance to either intact or deprived hosts in these infections. Differences in mean percentage parasitaemias (on days 2, 3 & 5) between deprived mice given normal AKR serum treated cells or anti-theta antiserum treated cells were not statistically significant.

DISCUSSION

Although the experiments reported here implicate both humoral and cellular factors in acquired resistance to P. b. yoelii they do suggest strongly that a cell associated component, rather than serum antibodies by themselves, might be of prime importance

in the host response to malaria.

Immune serum (IMS) from CBA mice which had recovered from a primary infection with P. b. yoelii was shown to confer little or no protection on non immune recipients. Hyperimmune serum (HPS) on the other hand, proved effective in protecting intact recipients against challenge inocula of 1×10^4 and 5×10^4, but when challenged with still larger inocula (1×10^5) it had only a temporary inhibitory effect on the infection. When HPS was administered to "deprived" recipients, it was found that doses of serum which protected intact mice completely, only caused a 5 to 6 day delay in the onset of infection. It had been demonstrated previously that these deprived mice could not mount an effective immunological response to P. b. yoelii, and the inability to respond appeared to be related, in part at least, to a failure of the germinal centre response and 7S antibody production. The ability of HPS to contain the infection temporarily in these mice suggests that the passively administered antibody effects a transient restoration of this immunological defect - the duration of which correlates well with the half life of such serum immunoglobulins (Fahey and Sell, 1965). In intact recipients too the passively transferred HPS probably exerts a similar inhibitory effect - thus providing the intact host with a sufficient interval of time during which it can mount a response against the parasite, thus preventing the infection from becoming patent.

When mice were treated with 0.5 ml of immune serum (IMS)

and subsequently challenged with either 1×10^4 or 5×10^4 parasitized cells, some enhancement resulted. This effect was more pronounced in the group challenged with the larger inoculum. Enhancement was also observed in mice challenged with 1×10^5 parasitized erythrocytes after treatment with HPS. The exact mechanism by which passively administered antibody caused such an effect in these infections is uncertain. However, in other situations such as the host tumour relationship, enhancement by passive antibody is thought to be due either to masking of target cell antigens, or to the inhibitory effects of antigen or antigen-antibody complexes on effector lymphocytes (reviewed by Hellstrom and Hellstrom, 1974). The possibility that essentially similar mechanisms might operate in the present situation cannot be overlooked.

Unlike serum antibodies, the transfer of immune spleen cells conferred very effective resistance in both normal and T-cell deprived mice challenged with an inoculum of 1×10^4 parasitized erythrocytes. Preliminary experiments (not reported here) have shown that the immunity transferred by these cells is as effective against larger challenge inocula, e.g. 1×10^5.

Mice which received immune spleen cells, showed initially, a more rapid development of the infection than normal cell recipients or untreated controls. Treatment of the cells with anti-theta antiserum or transfer into deprived recipients did not remove this effect. It seems likely that the immune cells or antibody produced by the cells

initially exerts a stimulatory effect on the parasite - thereby accelerating its growth.

Prehn (1971) suggested that the immune reaction to tumours might be biphasic - a mild reaction stimulating growth, and a strong reaction being cytotoxic. He found that when small numbers of immune spleen cells were mixed with tumour cells and inoculated into mice which had been "crippled" by thymectomy and x-irradiation, - tumour growth was accelerated. However, larger numbers of cells produced inhibition (Prehn, 1972). More recently Shearer et al. (1974) demonstrated that tumour cells exposed to antibodies specific for cell surface antigens multiplied more rapidly than control cells in vitro. Antibodies that were stimulatory at low concentrations were cytotoxic at high concentrations. It seems likely that analogous mechanisms could operate in the present situation. In fact, Prehn (Prehn and Lappe, 1971) predicted that if the immune response to a tumour proved to be biphasic, then a similar situation would obtain in relation to parasitism.

Treatment of immune cell donors with Cyclophosphamide - a process which is known to affect B-cells mainly (Turk and Poulter, 1972; Stockman et al., 1973) severely impaired the ability of immune cells to transfer resistance. Recent evidence suggests that Cyclophosphamide might also affect T-cell mediated immunity (Cole et al., 1972; Jokipii and Jokipii, 1973), the production of mononuclear phagocytes (Van Furth, 1974) and antibody dependent cell mediated cytotoxicity (Clark, 1974). Hence the lesion in these

animals may not be as specific as was originally suggested.

However, anti-theta antiserum treatment of the immune spleen cell population did not abrogate the capacity of these cells to transfer immunity, thus indicating that thymus processed cells were not directly involved in the adoptive transfer of immunity observed. This result is consistent with an earlier observation by Stechschulte (1969b), who found that thoracic duct lymphocytes (which consist of 80 to 90% T cells) from rats recovered from P. berghei infection lacked the ability to transfer immunity.

These findings indicate that the effector cells in the passive transfer experiments are non T cells. Such cells might be B axis cells or belong to the monocyte-macrophage series. These cells could exert their effects either by the production of humoral antibody, or by means of opsonic or cytotoxic mechanisms which are antibody dependent. However, as humoral antibody by itself is of limited effect in transferring immunity passively, when compared with immune lymphoid cells it seems unlikely that the role of such cells would be exclusively one of antibody production; rather, it seems likely that these cells might also subserve cytotoxic or opsonic functions.

The evidence for macrophage mediated opsonisation in malaria rests largely on the histological findings of Taliaferro and Mulligan (1937) who observed parasites in various stages of digestion within macrophages of the reticuloendothelial system, and on the more recent observations of Brown (1971) and Criswell et al. (1971).

Brown showed that in the presence of a weakly opsonizing serum, macrophages from BCG treated Parkes mice phagocytosed P. knowlesi infected red cells more readily than normal mice, while virtually no phagocytosis occurred in the absence of opsonizing antibody. Criswell et al. (1971) reported that the rate of destruction of parasitized cells implanted in Millipore chambers in P. berghei infected mice, was considerably increased if the Millipore chambers also contained macrophages. However, as both these workers used unfractionated peritoneal cells as their source of "macrophages", the results of these experiments can be interpreted differently. Further, the presence of parasites or parasitized cells within macrophages (Taliaferro and Mulligan, 1937), need not necessarily mean that opsonisation is the specific immunological process by which an infection is controlled. Opsonisation could be preceded by cytotoxic or antibody mediated processes which might damage or inactivate the parasite before opsonisation occurred. More recently Lourie and Dunn (1972) reported that rabbit anti macrophage serum (raised against adherent peritoneal exudate cells) had no effect on the course of a P. berghei infection in rats. Clearly it is important to examine the role of opsonisation in these infections in greater detail, possibly using better defined cell populations.

The non T effector cells might equally well exert their effects by a cytotoxic mechanism; the manner in which non T cells exert cytotoxicity has received much attention recently (see MacLennan, 1973; Forman and Moeller, 1973; Cerrotini and

Brunner, 1974). These cells have no affinity for target cell antigens as such, but are triggered into cytotoxic activity by antibody complexed to target cell antigens. The process is complement independent.

The precise nature of these non T effector cells is controversial. They are thought to be non adherent, non phagocytic cells, with receptors for the Fc part of antigen-antibody complexes (MacLennan, 1972; Moeller and Svehag, 1972; Perlmann et al., 1972) as well as receptors for complement (Perlmann et al., 1973; van Boxel et al., 1973); whether these cells are B cells, or belong to a so far unidentified population of non T cells has not yet been resolved. Greenberg et al. (1973) found that the effector cell was a non immunoglobulin bearing cell and suggested it was a member of the monocyte series, while Forman and Moeller (1973) have suggested that these cells are a sub-population of B cells, which possess surface immunoglobulin and are activated by LPS (Lipopolysaccharide) and SIII (Pneumococcal polysaccharide type III).

Although the phenomenon of antibody dependent cytotoxicity has been investigated largely using antibody coated target cells and normal lymphoid cells in vitro, it is thought that the lysis of untreated target cells by immune lymphoid cells operates in a similar manner (see Cerottini and Brunner, 1974). The mechanism would involve production of antibody by antibody forming cells, binding of this antibody to target cells, and the destruction of such antibody coated cells by effector cells carrying Fc receptors. (Hence for immune

lymphoid cells to be effective they must contain effector cells with Fc receptors and anti target cell IgG producing cells.) Evidence that immune lymphoid cells can exert such cytotoxic effects has come from a number of investigations (Van Boxel et al., 1972; Dennert and Lennox, 1972) although the effector cell has been variously identified by these workers.

Other lines of evidence lend further support to the possible occurrence of such a mechanism in malarial infections.

1. In rats, cell populations which are known to contain large numbers of these effector cells e.g. spleen cells, are the most effective in transferring immunity to P. berghei passively, while thoracic duct lymphocytes which contain many T cells and also some antibody forming precursor cells but no effector cells (MacLennan, 1973) lack completely the ability to transfer immunity to P. berghei (Stechschulte, 1969b). Further, the ability of an immune serum to transfer some measure of protection to non-immune rats is drastically reduced if the recipients are splenectomized (Brown, I.N. - personal communication) before serum transfer.

2. The transfer of immune spleen cells from rats recovered from P. berghei infection to non immune recipients leads to the formation of high levels of protective antibody in the recipients. Fractionation of these protective antibodies has shown they are largely of the IgG class (Phillips and Jones, 1972). The importance of the IgG type antibodies in malarial infections was further demonstrated in the present study where it was shown that while intact CBA mice

produced high levels of IgG_1 and IgG_2 antibody - the production of such 7S antibodies was severely depressed in "deprived" mice which always succumbed to a P. b. yoelii infection. The production of IgG antibodies is thymus dependent and their role in antibody dependent cell killing could account for the overall thymus dependency of the immune response in malaria.

3. Depletion of C^3 in rats, does not affect the immune response to P. berghei infection (Diggs et al., 1972), hence the immune mechanism is probably complement independent.

Although the results presented here and other evidence cited suggests that antibody dependent cell killing might be operative, it does not constitute unequivocal evidence for the participation of this mechanism in malarial infections. The importance of IgG antibody and the occurrence of synergism between such antibody and non T effector cells might well be interpreted in terms of an opsonic rather than a cytotoxic mechanism. It would seem necessary to carry out definitive experiments to differentiate clearly between these two possibilities; particularly important would be to determine whether unprimed, T cell depleted lymphoid cell populations can exert cytotoxic effects on parasitized RBC, in the presence of antibody.

Even though non T cells appear to be the main effector cells in the passive transfer of resistance from recovered mice to non immune recipients this does not rule out the possibility that T cells could also play a cytotoxic role earlier in the infection. It was

demonstrated using the T6T6 chromosome marker that infection with P. b. yoelii triggered off a vigorous mitotic response in the T cell pool. Although the significance of such mitosis is uncertain it could, in part, be indicative of cytotoxic activity in the T cell pool during an active infection. Such a pattern of responses, i.e. activity in the T cell pool early in the response followed by a shift of such activity to the non T cell pool, would be in accordance with the cellular changes seen in the spleen, and has been reported in other situations as well. Notably, Lamon et al. (1972) who studied the nature of cytotoxic cells in mice bearing Moloney Sarcoma Virus (MSV) induced tumours, found cytotoxic T cells just prior to tumour development and soon after regression, but later on cytotoxic activity was mainly confined to the non T cell compartment.

It must however be remembered that malaria parasites are present in the peripheral blood, both as intracellular and extracellular forms (merozoites). The immune mechanisms which operate against the two stages could be fundamentally different. While cellular mechanisms of the type discussed could be of prime importance in attacking the intracellular stages, it is conceivable that humoral factors might be more important in immune mechanisms directed against the merozoites. Cohen's observation (Cohen et al., 1969) that serum antibody inhibited parasite replication in vitro by preventing merozoites from invading RBC would be consistent with such a hypothesis.

CHAPTER 6

GENERAL DISCUSSION

This study was initiated to define certain fundamental aspects of the host parasite interaction in murine malaria. Although many basic features of the host response have been revealed, there is clearly much that needs to be studied, before the ultimate goal of immunological investigations - effective immunization - can be achieved. In this chapter, the discussion of certain features of the host response will be extended, and an attempt will be made to indicate those aspects of the host parasite interaction which need to be analysed further, to obtain a more complete understanding of the immune response to this infectious agent. Some of the discussion will necessarily be speculative, but it is hoped that such speculation will provoke further experimentation and testing out of the ideas presented.

The murine malaria parasites, like many other infectious agents, appear to provoke a wide range of responses in the host, but the expression of immunity at any one point is probably critically dependent only on a few of these responses. The responses provoked include effector responses directed against the parasite and suppressor responses which mediate non specific suppression of the immune response to other antigens, and in some instances also seem to suppress the response to the parasite itself. The outcome of any particular infection therefore appears to be dependent on a balance between such effector and suppressor responses.

EFFECTOR RESPONSES

Various lines of evidence indicate that in the mouse, T cells play a central role in effector mechanisms directed against the parasite. T cell deprived mice are unable to generate an effective response to P. b. yoelii while anti thymocyte serum (ATS) treatment also seriously impairs the host response. Reconstitution of T cell deprived mice with syngeneic, chromosomally labelled thymus grafts results in restoration of immune responsiveness, and the labelled T cells show a vigorous proliferative response during P. b. yoelii infection. Mice reconstituted to different levels of immunocompetence with thymus grafts show corresponding degrees of immune responsiveness to the parasite. Hence, there is little doubt that T cells are essential for the generation of an immune response. On the basis of histological and serological observations, and the results of passive transfer experiments it seems clear that this T cell dependency is at least in part related to antibody production, and that this T-dependent antibody acts synergistically with a non T cell in an effector response directed against the parasite. However there are certain aspects of the T cell response which need to be examined more critically.

First, it is imperative to determine unequivocally whether T cell activity in these infections is concerned exclusively with antibody production, or whether an antibody independent direct T cell cytotoxic component is also involved in controlling an infection. It would be useful to carry out direct cytotoxicity testing with

fractionated cell populations (using ^{51}Cr release or the microplate assay) both during and after recovery from a P. b. yoelii infection. The failure of Phillips et al. (1970) to demonstrate a cytotoxic effect on P. knowlesi parasites with sensitized lymphocytes need not necessarily imply that such mechanisms are lacking in all malarial infections. P. knowlesi is known to cause a fulminant infection in the rhesus monkey killing it in 8 to 10 days. Hence this infection might inhibit T cell activity or, if in fact T cell cytotoxic activity is elicited only during an active infection it may not have been detectable in the drug treated recovered animals used in these experiments.

It would also seem important to determine the precise nature of the targets against which T cell dependent effector responses are directed. It is not known at present whether all erythrocytic stages of the parasite are equally susceptible to the effects of immunity, or whether immune responses are directed specifically at any particular developmental stage. A number of workers have suggested that the mature forms of the parasite are more sensitive to the effects of immunity (reviewed by Brown, 1969) but there is no direct experimental proof that protective immune responses are directed against these stages. Certain observations on the so called "crisis forms" of the parasite are perhaps relevant in this connection. In P. floridse (Thompson, 1944) and P. brasilianum (Taliaferro and Taliaferro, 1934) infections, at the time of crisis - when an antiparasitic response becomes apparent -

a marked reduction in the number of merozoites per schizont has been observed; while in P. ovale an increase in merozoite numbers has been reported in humans and chimpanzees which relapsed after splenectomy (Adler and Zuckerman, 1952; Bray, 1957). Further, large vacuoles and altered staining reactions to Romanowsky stains in the mature forms of P. floridse were observed at the time of crisis.

These observations could mean that some component of the immune response acts by interfering with parasite reproduction during schizogony. Antibody alone is thought not to affect the intracellular stages of the parasite (Cohen et al. , 1969), but antibody mediated effects on the dividing parasite could be preceded by cytotoxic responses. It is possible that initially the host RBC (containing a mature parasite - possibly an early schizont) rather than the parasite itself, is the target of cytotoxic mechanisms directed against antigens expressed on the surface of the parasitized cell. Such cytotoxic responses (possibly mediated by T cells during the earlier part of an infection and by non T cells later, and on recovery from a primary infection) might not be effective by themselves but could serve to expose the mature parasite to the external environment. If this environment contained high levels of antiplasmodial antibody, the antibody could enter the damaged red blood cell and interfere with the asexual phase of multiplication.

It was also observed during this investigation that immune lymphoid cells, initially appeared to have a stimulatory effect on

parasite growth - an observation in agreement with Prehn's immunostimulation hypothesis (Prehn, 1971a). If in fact a low level of immune reactivity stimulates parasite growth it would have important implications in persistent malarial infections. It might also be indicative of a fundamental difference between weakly and strongly antigenic parasites. While the former would elicit an immune response that stimulates parasite growth, the strongly antigenic parasites would evoke a response which inhibits growth.

SUPPRESSOR RESPONSES

These infections also appear to trigger off a series of suppressor mechanisms which reduce host immunological reactivity. It was suggested in earlier sections of this thesis that various factors such as antigenic competition, infiltration of T dependent areas and consequent changes in cell relationships, elevated steroid levels and thymic atrophy, and direct inhibition of T cell function might be responsible for some of the suppressive effects observed. Clearly it is important to study these and other relevant mechanisms in greater detail, and define more precisely the manner in which they mediate their suppressive or regulatory effects.

For instance antigenic competition is probably of considerable importance in the transient non specific suppression of immune function observed in P. b. yoelii infections. However, the effects of antigenic competition could be due to a number of different factors (reviewed by Pross and Eidinger, 1974). Much attention

has been focussed recently on the possibility that in a competitive situation specific suppressive factors may be released by activated T cells (see Pross and Eidinger, 1974) or suppressor T cells (Gershon, 1974). It would be important to determine whether such mechanisms operated in malarial infections.

Also relevant to the depressed T cell reactivity observed in these infections is the work of Scott (1972a & b) on Coryne bacterium parvum treated mice. These mice showed severely depressed PHA responses in spleen and peripheral blood, and certain other T cell functions were also depressed. Scott has presented striking evidence that the inhibition of T cell responses is due to increased activity and possibly a qualitative change in the macrophage population. Malarial infections are also known to cause increased macrophage activity as judged by carbon clearance in mice (Greenwood et al., 1971) and rats (Elko and Cantrell, 1970). It would be useful to examine the possible implications of such hyper-activity in the light of Scott's observations.

It was demonstrated during this investigation that P. b. berghei infections elicited limited T cell mitotic activity, and that this activity faded completely despite a persistent infection. It was suggested that the non specific failure of immunity observed in these infections might have been secondary to a specific failure of immune responsiveness to the parasite. It is necessary to determine whether this failure of immunity was merely due to the inability of the parasite to induce an effective T cell response, or whether in fact the parasite actively "switched off" T cell function.

The possibility that some form of antigen induced paralysis or blocking of lymphocyte function may have been responsible was discussed earlier. In the tumour situation (where direct T cell cytotoxic responses are the main effector mechanisms) it is now established that serum factors (antigen-antibody complexes, antigen, or antibody) can block T cell responses (reviewed by Hellstrom and Hellstrom, 1974). Either an efferent form of blocking in which there is blindfolding of target cell antigens or a more central form in which T cell activity is blocked by antigen-antibody complexes or antigen alone is thought to occur, but the latter form of enhancement appears to be the more important in the host tumour relationship.

The importance of analogous mechanisms during malarial infections and their significance as escape mechanisms for the parasite have not been examined in any great detail. However, Jerusalem et al. (1971) reported that Swiss mice developed considerably enhanced P. berghei infections if the mice were repeatedly vaccinated with non viable antigen prior to infection. Mice treated with anti thymocyte serum (ATS) during immunisation did not develop enhanced infections. Blocking of effector lymphocyte function might be one of the methods used by malaria parasites to inhibit immune responses. Wilson's (reviewed by Wilson, 1974) demonstration of large quantities of soluble antigen during P. falciparum infection is interesting in this connection, since such antigen acting either alone or complexed with antibodies might be involved in the blocking of

lymphocyte responses. Alternatively, soluble antigens might induce the proliferation of suppressor cells which may exert direct suppressive effects on the response to the parasite.

Suppressor cells may also be involved in the apparent "protective" effects of malaria on the development of autoimmune disease in the NZB mouse (Greenwood and Voller, 1971). It is now thought that most autoantigens which circulate in low doses induce unresponsiveness in specific populations of T cells but not B cells (Allison, 1971; Weigle, 1971). It has been suggested that NZB mice are specially prone to autoimmune disease because their T cells are resistant to tolerance induction, and that T cell functions in these mice (including regulator and suppressor functions) decline rapidly with age. Gershon (1974) has discussed the possibility that both T cell helper and suppressor effects are mediated by T_2 type cells, and that NZB mice are largely incapable of functions ascribed to T_2 cells. It is tempting to speculate on the possibility that the ability of malarial infections to markedly delay the onset of autoimmune disease, might be due to the parasite triggering off the production of suppressor elements in which these mice appear to be deficient.

CONCLUSIONS

It can thus be seen that while T cells are crucial for the generation of a protective response against the parasite, the parasite itself can trigger off a range of mechanisms which damp down T cell activity, resulting in either a non specific depression of responsiveness

to certain antigens or possibly a specific suppression of the response to the pathogen itself. Hence it would appear that the ability of a parasite to suppress or modulate T cell dependent effector responses is fundamental to its survival in the host. This situation does not appear to be unique to malaria. It is likely that broadly similar mechanisms operate in diseases such as Lepromatus Leprosy and Diffuse Cutaneous Leishmaniasis (DCL), the severe forms of these spectral diseases. A greater understanding of factors which stimulate and suppress T cell activity in malarial infections and their relevance to the development of protective immunity is vital for a more complete understanding of the host parasite interaction, and an essential prerequisite before effective immunisation is achieved; a vaccine which fails to stimulate a T cell response would be of little value if the development of resistance depended critically on such a response.

There is clearly much work to be done on the immunology of malaria before this disease is brought under control, and it is likely that the murine malarial models used in this investigation will play a significant role in future developments in this field. It is heartening to note the increasing awareness of the dimensions of the problem presented by malaria and other parasitic diseases, and of the potential inherent in the application of the newer immunological concepts to these parasitic diseases. The increasing number of immunologists turning their attention from the theoretical complexities of the immune response to the equally challenging problems presented

by the parasitic diseases augurs well for Parasitology. Perhaps, the first and second "Golden Ages of Thymology" (Miller, 1967) are drawing to a close, but the "Golden Age of Parasitology" is only just beginning.

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