THE ROLE OF CELL INTERACTIONS IN LYMPHOCYTE CIRCULATION

Miles D. J. Davies B.Sc.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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This thesis is dedicated in memory of my father.

DECLARATION

Parts of this thesis have already been published in the following papers.

Evans C. W. & Davies M. D. J. (1977)

The influence of cell adhesiveness on the migratory behaviour of murine thymocytes.

Cellular Immunology 33 211-218

Curtis A. S. G., Davies M. & Wilkinson P. C. (in press 1978)

New evidence on control mechanisms in lymphocyte traffic.

To be published in "Proceedings of the Sixth International Conference on lymphatic tissues and germinal centres in Immune reactions"

Other parts have been presented as poster demonstrations at symposia of the British Society for Immunology and the Society for Experimental Biology.

ABBREVIATIONS

Although quoted in the text, abbreviations are also listed here.

AF	aggregation factor
ALS	anti-lymphocyte serum
ARC	Agriculture Research Council; animal supplier
Bact. & Imm.	University of Glasgow Department of Bacteriology & Immunology; animal source
B & K	Bantin & Kingman; animal supplier
B lymphocyte	thymus independent, bone marrow-derived lymphocyte
Con A	concanavalin A
cpm	counts per minute
CPP	cyclophosphamide
DAH	differential adhesion hypothesis
DNase	deoxyribonuclease
EDTA	ethylenediaminetetraacetic acid
EPM	electrophoretic mobility
FCS	foetal calf serum
GALT	gut associated lymphoid tissue
g	grams weight
g.	gravitational force
HCR	hydrocortisone resistant
HEV	high endothelial venule
Ig	immunoglobulin
IMF	interaction modulation factor
LN	lymph node
LNL	lymph node lymphocyte
LPS	lipopolysaccharide

M	molar
µg	microgram
mg	milligram
ml	millilitre
MLN	mesenteric lymph node
mM	millimolar
mOsmol/kg	milliosmoles per kilogram
N	normal solution
nm	nanometer
PL-A	phospholipase A
PL-C	phospholipase C
PLN	peripheral lymph node
PML	polymorphonuclear leucocyte
PNA	peanut agglutinin
R	rads. (measurement of radiation)
rpm	revolutions per minute
RPMI	Rosewell Park Memorial Institute
SEM	scanning electron microscopy
SM-C	Sphingomyelinase C
T lymphocyte	thymus-derived lymphocyte

SUMMARY

It has been known for some time that filtration of a heterogeneous population of lymphocytes through a nylon wool column will produce an eluate containing almost exclusively T lineage lymphocytes. Results presented in this thesis show that filtration of thymus lymphocytes through nylon wool produces a subpopulation which exhibits both a greater mutual adhesiveness and a temporary difference in localisation patterns when injected back into syngeneic recipients as compared to the unfiltered population. Further analysis of the adhesiveness and localisation patterns of filtered and unfiltered T lymphocytes derived from lymph nodes was used in an attempt to support the hypothesis that the in vivo localisation characteristics of a lymphoid cell can be predicted from its in vitro adhesiveness to like cells. The data, however, was not compatible with this concept which was rejected as being too simple.

It was found that the localisation of particular radioactively labelled lymphoid cells could be disrupted by the simultaneous injection of a large number of unlabelled lymphoid cells of a homogeneous lineage. This effect is not thought to be the result of physical obstruction by the extra cells but rather due to substances released by the unlabelled cells which alter the interaction of the labelled cells with resident and circulating cells within the lymphoid system.

The presence of substances released by lymphocytes which can modulate the interactions between lymphocytes of the opposite lineage has been previously reported. The effect of these substances, interaction modulation factors (IMFs), on the localisation of injected labelled

lymphoid cells was studied and the results suggested that the effects seen after treatment of injected labelled cells with large numbers of unlabelled cells could possibly be attributed to the release of large amounts of IMFs by the latter cell type.

The stages of lymphocyte circulation and their possible control mechanisms are discussed in depth but none can adequately explain the overall positioning patterns that are displayed by lymphoid cells. The action of IMFs in vivo is put forward as an overall control mechanism of lymphocyte circulation and is compared with other theories which have been suggested to explain the specific sorting-out of cells in embryonic and morphogenetic systems.

To doubt everything or to believe everything are two equally convenient solutions; both dispense with the necessity of reflection.

Jules Henri Poincaré (1854-1912)

CHAPTER ONE

INTRODUCTION

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INTRODUCTION

The necessity for a cell to be able to specifically migrate from one point in an organism to another is fundamental to development and survival (see Trinkaus 1969). Stem cells in the embryo must be able to populate and proliferate in particular organs, but migrational specificity is also crucial in adult life as is evident from the movements of certain motile blood cells. The exact positioning and functioning of these cell types is essential in the body's constant fight against disease and any deviations from the normal have disastrous consequences.

The presence of active motile blood cells has been known for almost a century due to the pioneering studies started by Virchow and Metchnikoff. However, the majority of the observations on these cell types, at least until thirty years ago, was the result of painstaking work with the light microscope and often little consideration was given to the correlation with function and movement in vivo. The polymorphonuclear leucocytes (PML) were known to be able to phagocytose foreign objects in vitro but how they recognised and acted on such objects in vivo was a complete mystery. The knowledge of lymphocytes was also vague; they were known to be involved in the body's immune system, but, in the absence of our present knowledge of the immunoglobulins, their action remained unknown.

During the 1950s, it was realised that white blood cells had an innate ability to home to active or inflamed regions while under normal circumstances, some of these cells remained in vascular circulation, moved passively by fluid flow and the remainder were stationary within particular organs.

Today, we know where almost all white blood cells migrate to, what can stop their migration, what can enhance it, what they do when they reach a particular place and generally all there is to know about the "private life" of such a cell. What we do not know is what happens in the "mind" of the cell - why, under normal circumstances with no infection, should a cell circulate and not become trapped in organs along with similar, though not identical compatriots. The theory has been put forward that lymphoid cell recirculation occurs to better the chance of a particular antigen-sensitive cell meeting a specific antigen on its presentation. This is a feasible suggestion since only one in a million cells will be sensitive to a particular antigen (Ford 1975). However, the fact that recirculation occurs in the foetus, which is immunologically immature, makes this theory slightly dubious.

In this particular study, the role of intercellular adhesion as a possible control mechanism in lymphocyte traffic will be discussed in the light of present and past experiments. That these control mechanisms have implications in the aetiology of certain diseases is particularly evident in the case of mycosis fungoides. This

condition is manifested by a selective migration of one lymphoid cell type to the skin resulting in bizarre dermatological disorders and immunological defects resulting from the loss from circulation of that cell type (Goos et al 1976).

CHAPTER TWO

THE PROPERTIES AND SEPARATION OF B AND T LYMPHOCYTES

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For many of the localisation experiments undertaken, it has been necessary to attempt to produce cells of a homogeneous lineage, i.e. T or B. In this chapter, some of the major differences between T and B cells will be reviewed with reference to their separation and the validity of using the separated cells for circulation studies.

That there are two lineages of lymphocytes in mammals has been known for some time, although the classification into T and B types was only made fairly recently (Roitt et al 1969). However, the ontogeny of these two types remains obscure. Lymphocytes probably arise from multipotential stem cells originally in the yolk sac which migrate to the foetal liver and bone marrow and then to the primary lymphoid tissues to be subsequently processed (Stutman 1976). Cells migrating to the thymus (Harris et al 1964) develop T cell characteristics (Stutman & Good 1971), but only a small number of these cells ever develop further into peripheral T cells and migrate from the thymus (Bryant 1971). The latter cell type probably arises in the thymic medulla where a small population (3 - 5%) has been found which is relatively insensitive to the effects of radiation and corticosteroids (Blomgren & Andersson 1969, 1971, 1972) and shares many attributes characteristic of peripheral T cells (Raff 1971, Raff & Owen 1971). Since the majority of thymocytes are rapidly dividing (Metcalf & Wiadrowski 1966, Joel et al 1977), those cells

not destined to become peripheral T cells must either die in situ or migrate from the thymus to be reprocessed or die elsewhere (Bryant 1971). It has recently been suggested that the differentiation of T cell subclasses is not due to sequential stages of a single pathway, but rather due to separate lines of differentiation arising in early foetal life (Goldschneider 1976, Huber et al 1976, Papiernik et al 1977).

The processing of B lineage lymphocytes in mammals is not well understood at all. In birds, stem cells migrate into a gut-associated primary lymphoid organ, the Bursa of Fabricius, wherein they are processed, followed by migration in a mature form to peripheral tissues (Moore & Owen 1966, Durkin et al 1971, de Kruyff et al 1975). That B cells are found in the avian thymus and T cells in the Bursa has presented an as yet unexplained problem. Mammals, however, possess no organ which has definitely been assigned "Bursal" functions although both foetal liver and bone marrow are known to give rise to stem cells which can differentiate in situ to form B lymphocytes. Owen et al (1977) have described a pathway of cells found in foetal liver and bone marrow showing cytoplasmic IgM which develop into cells exhibiting surface IgM (characteristic of more mature B cells) in the bone marrow only. Adult bone marrow contains very few fully differentiated B cells (Basten et al 1971), but still contains many IgM positive cells which can further develop into mature B cells which are IgG or IgA positive (Osmond et al 1976). Most of the gut associated lymphoid tissues (GALT)

have been suggested at one time as having "Bursal" functions (reviewed in Greaves et al 1974). In the rabbit, Peyer's patches, aggregates of lymphatic nodules along the length of the small intestine, have been suggested as being a Bursal equivalent from studies after their surgical removal (Faulk et al 1971), but since this technique requires major surgery on the experimental animal, a large disruption of the lymphoid system status quo would inevitably result. Another observation on Peyer's patches has suggested that they show the characteristics of peripheral rather than central lymphoid tissue (Perey & Milne 1975). Thymus cells are known to migrate to the Peyer's patches of newborn mice (Joel et al 1971) and to the GALT of young rabbits (Barg & Draper 1975) and they have been found to contain all the known T cell subsets which act in the Graft-versus-Host reaction (Vuitton et al 1977). Thus, although it seems that Peyer's patches can hardly be a central B cell processor due to their T cell function and content, Perey et al (1968) found that removal of the appendix, sacculus rotundus and Peyer's patches, followed by lethal irradiation and foetal liver reconstitution, in rabbits abolished the antibody response to certain antigens. This observation may, in fact, argue for a possible "Bursal" function of the appendix, but a study by Nieuwenhuis (1971) found no evidence to suggest that it might be a central lymphoid organ. Clearly, then, the GALT as a whole have some role in B cell development, but at the present time there is little more definite evidence.

Although all lymphocytes have a common origin, the subsequent processing in the central lymphoid organs produces cells with very different physical, chemical and biological properties. These differences allow easy identification of each type and provide the basis for many separation techniques. The classification of cells into T and B as discrete categories is somewhat misleading and applies rather more to the developmental pathway than the finished product. Mature B cells have been found with characteristics which are usually attributed to T cells and vice versa, not unlike the division between female and male as sexes, who, although developing in a similar way, have diverged into the two forms which nevertheless retain characteristics of both types.

One aspect which exhibits this feature is the electrophoretic mobility (EPM) of the various lymphocyte types. The EPM is a function of the overall charge on the cell and is mainly due to the chemical make-up of the cell surface components. The thoracic duct lymph (TDL) of rodents contains 70-90% of cells classified as T lineage (Raff 1969), and studies on the EPM of TDL lymphocytes prior to this work (and the original classification by Roitt et al (1969)) showed that there are two electrophoretically separable populations of small lymphocytes (Ruhenstroth-Bauer & Lücke-Huhle 1968). They found that 77% of cells (designated type B, though confusingly not B lymphocytes) had a consistently higher EPM than the remaining 23% (type A). Moreover, type A lymphocytes were larger than type B, (although they were all classified as small lymphocytes),

were depleted by whole body radiation of 800R, and were directly correlatable numerically with the number of antibody producing cells. This suggests that type A were B lymphocytes and type B were T lymphocytes. This point has been confirmed by many other workers (e.g. Nordling et al 1972, Sundaresan et al 1975). One interesting point was that blood was found to contain almost all type A cells (B lymphocytes) whereas other evidence from surface markers suggests that the majority of blood lymphocytes (about 75%) are T type cells (Raff 1969, Vassar et al 1976). Clearly then, although the EPM of a cell is a rough guide to its lineage, the use of the technique of preparative cell electrophoresis to produce homogeneous T and B populations is not entirely valid although used in the past (von Boehmer et al 1974, Zeiller et al 1972).

The advent of the scanning electron microscope (SEM) was hoped to provide many of the answers about the ultrastructural details of the cell surface. This may undoubtedly be so in some fields, but in the realm of lymphocyte biology it has led to some very fierce controversy. In a study of the surface morphology of murine B and T cells (Polliack et al 1973, 1975), it was stated that T cells were "smooth" and B cells "villous". This finding was confirmed by Padnos (1976) using Hoffman modulation contrast microscopy with cell suspensions and smears, supposedly involving less handling of the cells likely to cause artefacts. However, similar SEM studies on human and murine cells failed to reveal such differences (Alexander & Wetzel 1975, Alexander et al 1976, Newell et al 1976)

and suggested that the preparation technique produced the "smooth" cells. It is likely that lymphocytes undergo continuous changes of surface morphology as they mature, divide, circulate and interact with other cells during normal in vivo functioning (Alexander et al 1976). In a particularly elegant study of lymphocytes migrating through the high endothelial venules (HEV) of lymph nodes, van Ewijk et al (1975) concluded that recirculating T and B cells have numerous microvilli which are necessary for the initial interaction with the HEV cells. Once diapedesis between the cells begins, the microvilli are withdrawn, only to reappear when the cells are back in circulation. However, Loor & Hägg (1975) found villi on recirculating and sedentary cells, contrary to van Ewijk's results, and also that sodium azide did not inhibit the villi which were suggested to be microfilament supported. These results are particularly interesting in the light of experiments by Ford et al (1978) on the mechanism of passage of lymphocytes through HEVs and will be discussed in a later section. Whether the villousness of the cells is that significant remains to be seen, but certainly the concept of different degrees of villousness on B and T cells finds some support from the nylon wool separation experiments discussed later.

Obviously the best methods of separation (or rather enrichment) of lymphocyte sub-classes utilise the different surface components. In many instances this has involved the selective retention of B cells on some type of substrate leaving the T cells unbound. On B cells the presence of surface-bound immunoglobulin, receptors for

C3-complement and the Fc units of antibodies has made this problem easier. T cells display a paucity of known surface antigens; in rats and mice, a brain-associated T cell specific antigen designated Theta has been reported (Reif & Allen 1964) which has subsequently been renamed Thy 1 and is a glycoprotein of 25,000 daltons molecular weight (Letarte-Muirhead et al 1975). However, in a recent report it has been suggested that B lymphocytes in the rat are derived from Thy 1-positive cells in the bone marrow with the result that this antigen cannot be reliably used as a marker for T lineage lymphocytes, at least in the rat (Hunt et al 1977). A Thy 1-type surface antigen has been isolated in humans but does not appear to be a marker for thymus lymphocytes and is found mainly on thymic epithelial cells (Arndt et al 1978). The recent discovery of the Ly antigen series may also provide a useful basis for subsequent cell separation (Cantor & Boyse 1975). Separation techniques utilising surface antigens have involved the selective binding of B cells to anti-immunoglobulins bound to polymethylmethacrylate beads (Basten et al 1972, Campbell & Grey 1972, Wigzell & Andersson 1969) or Sephadex beads (Chess et al 1974) although the latter has been criticised due to the fact that passage of B cells through the column may cause surface immunoglobulin and Fc receptors to become masked and thus remain undetected in the eluate (Karpf et al 1975). However, in a more recent report, the use of C3-complement linked to Sepharose did not encounter such a problem (Casali & Perussia 1977). These separation methods supposedly produce pure populations of T lymphocytes leaving the B lymphocytes bound (they can

usually be eluted but often become damaged in the process) but the large quantities of antisera needed for the columns makes it an inviable technique in a small laboratory. No anti-T cell column purifications of B cells appear to have been widely used.

One of the most extensively used but least understood of the separation techniques involves the use of nylon wool columns which specifically retain B lymphocytes (Bianco et al 1970, Julius et al 1973). The specificity of the technique is extraordinary in that from a heterogeneous input, the eluate contains around 95% T lineage cells. How the selective retention works is a mystery, although the suggestions from SEM work that B cells are more villous than T cells, and hence are trapped, may have some bearing on the mechanism. Subsequent removal of the B cell population has been achieved by mechanical disruption of the column although the cells have a low viability and are very mixed in lineage (Trizio & Cudkowicz 1974, Handwerger & Schwartz 1974) but the use of disaggregation agents such as calcium- and magnesium-free medium or EDTA failed to release the bound cells (C. W. Evans, personal communication). The fact that the technique is temperature and serum sensitive indicates an active process, although incubation of the columns in the cold after elution of the T cell population does not cause the release of adherent cells (personal observation). A further twist to the mystery was added in the report that passage of thymocytes through a nylon wool column produced a subpopulation with a greater mutual adhesiveness than that of the input population (Evans & Davies 1977). This suggested that when used for the separation of B and T cells, some T cells might remain bound to the columns as

well, and that the eluted subpopulation might be a specific sub-class of T cells. Although Wiig (1976) found this proposition to be false from the point of view of T lymphocyte localisation patterns, the results of work reported in this thesis conflict with his findings. Thus, although the technique is quick, cheap and efficient in the purification of T cells, the resultant population should not be expected to contain a full complement of T cell subsets and thus, care should be taken in subsequent interpretations.

Other separation techniques have involved the binding of B cells via their complement receptors to a solid phase (usually sheep red blood cells) and their subsequent removal by differential centrifugation; separation of fluorescently labelled subsets in a fluorescence-activated cell sorter (Cantor et al 1975); by specific agglutination of one cell type with lectins (e.g. Reisner et al 1976); density gradient centrifugation (Bøyum 1968, Morrison 1967, Raidt et al 1968, Tse & Dutton 1976) and a host of more or less complex methods (reviewed by Hunt 1978). The type of separation to be used depends on the experiment - in the instance of recirculation experiments, the best source of T cells is from thoracic duct lymph (70-95% T cells) and B cells from adult thymectomised, irradiated and bone marrow reconstituted animals. As the former technique in mice is extremely difficult, some of the separation techniques described here have to be used, and interpretations using these cells must be made with discretion in the light of the problems mentioned.

CHAPTER THREE

RECIRCULATION AND ITS CONTROL MECHANISMS

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The words migration, localisation, recirculation, traffic, homing and ecotaxis are among the many found in the literature concerning cell movements which have similar meanings. At the outset of this appraisal of the knowledge of lymphocyte movements, it is as well to define each term as it will be used here.

Migration, as referred to the movement of neural crest or primordial germ cells in the embryo or neutrophils to a site of inflammation in the adult, implies a movement from one site to another, the latter site being more or less permanent. However, migration as applied to birds suggests, as in their migration from Britain to Africa, a degree of transience as the majority of birds will return to Britain at the start of the season, a feature which has more in common with the recirculation of lymphoid cells, shortly to be described. Thus, neural crest cells migrate to various different areas to make up, amongst other organs, parts of the adult nervous system and teeth (Chibon 1967), primordial germ cells migrate to prospective gonad areas (Meyer 1964) and neutrophils migrate into areas of inflammation (Grant 1973) - all one way movements. The migration of lymphocytes in this instance will be regarded as a part of the process of recirculation, a temporary stop for cells en route. The use of the word homing in lymphocyte studies has been a mistake. By definition, homing means to return to the normal place of residence, a feature not normally exhibited by cells. Chick embryo

cells, when introduced into the venous system, failed to home to their tissue of origin (Burdick 1968). Lymphocytes are often described as "homing to the lymph nodes" but this usually means "migrating to the lymph nodes". Ecotaxis, coined by de Sousa (1971), implies more "discrimination" on the cell's behalf - i.e. thymus cells in circulation will localise preferentially in thymus-dependent areas of the lymphoid tissues. However, since it may be the interactions between cells, rather than the action of the cell itself, that governs this process, the use of the term ecotaxis may not be entirely correct.

Recirculation is the movement of a cell from one point back to the same point via various different organs within a short space of time like a car being driven around an urban ring road. Thus, recirculating lymphocytes in the blood migrate through lymph nodes into lymph and return back to the blood in the course of a day or so, thence to repeat the process once more (e.g. Sprent 1973). As far as is known today, the process of recirculation as described is unique to lymphoid cells and requires a high degree of selectivity for the migration of only certain classes through the lymph nodes.

In the results presented in this thesis, localisation is used to describe the number of cells to be found in a particular organ after a particular time following intravenous injection. It does not imply any degree of recirculation or ecotaxis as there is insufficient evidence from this type of experiment to draw any further conclusions.

Overall, the movement of any cell type, by any means to any place, can be called traffic. This chapter will deal specifically with lymphoid cell traffic and some experiments which may throw light on its control.

To retrace the history of experiments leading to our present knowledge of lymphocyte recirculation would be pointless when many modern reviews are available which cover the subject in more detail than would be possible here (Greaves et al 1974, Ford 1975, de Sousa 1976, Sprent 1977). However, certain key and more recent experiments will be mentioned to pave the way for further discussions.

The majority of studies on lymphocyte recirculation was initiated by the classical work of Gowans and co-workers (Gesner & Gowans 1962, Gowans 1959, Gowans & Knight 1964) on the output of small lymphocytes from the thoracic duct of rats and mice with indwelling cannulae. The results of these experiments showed that the output of small lymphocytes is maintained by the recirculation of cells from the blood to lymph through lymph nodes and Peyer's patches. The time taken for a cell to circulate from blood to lymph (essentially the blood to blood time) varies between 12 and 36 hours in mammals and rather quicker in other vertebrates (de Sousa 1976). Although the concept of blood-lymph as the major route of recirculation is almost universally accepted, it has been suggested (at least in the dog) that most cells entering the lymph node arrive via the afferent lymph rather than from the blood (Sainte-Marie et al 1975) although Hall & Morris (1965) found more lymphocytes in efferent than afferent lymph which suggests that the excess is due either to an influx of

recirculating cells from the blood or from cell division within the lymph nodes. Both these explanations are probably true, but the crux of the argument put forward by Sainte-Marie (1975) is that Gowans & Knight (1964) did not produce enough evidence to prove that high endothelial venules (HEV) are the sole site of entry of lymphocytes into the nodes to account for the numbers of cells appearing in the thoracic duct lymph, amounting to an average of about 3.5×10^6 cells/hour in the mouse (Boak & Woodruff 1965). The problem of the explanation of cells arriving from the afferent lymph is the origin of these cells - afferent lymph drains tissues but it is highly unlikely that lymphoid cells migrate from blood outside the blood-lymph circuit since very few lymphoid cells migrate from blood to non-lymphoid tissues (Rannie & Donald 1977) and cells, once outside the blood-lymph system, very rarely return to circulation, at least under normal conditions when the cells are not stimulated (Hollingsworth & Carr 1972).

Perhaps the most pertinent question of all concerning recirculation is why it should occur in the first place. The explanation usually given is an essential part of the interpretation of the clonal selection theory of antibody production put forward by Burnet (1959). This theory was suggested to explain how a rapid antibody response could be initiated towards an antigen never before encountered by the immune system. It was supposed that there are, in the lymphocyte system, cells which are sensitive to a particular antigen (why they are sensitive is another problem), probably amounting to about one cell in a million. Contact with antigen would cause this cell to

proliferate into a clone of antibody producing cells. However, in a system of static lymphocytes, the chance of an antigen meeting a cell sensitive to it is minimal, but if the cells were able to recirculate throughout the blood-lymph system, then the chance would be much greater. De Sousa (1976) argues that since the blood circuit itself covers all parts of the body, then recirculation from blood to lymph is unnecessary - as demonstrated by the pig which has a poorly developed lymphocyte circulation system (Binns & Hall 1966) and yet can mount normal primary and secondary antibody responses. Since the pig has anatomically unique lymph nodes, then there is some reason to believe that it might be a special case. The assumption that the blood system covers the whole body is also not entirely true since it will not enter the actual tissue of a lymph node itself, the expected site of trapping of a non-blood borne antigen (Zatz 1976a). Further evidence for this suggestion is that the sequestration of lymphocytes by antigen in the spleen (originally blood-borne) is brought about by different cells and by a different mechanism to that in the lymph nodes (Zatz 1976a & b). Thus, for a complete dissemination of immunological ability, recirculation would seem to be necessary, but this suggests that only cells carrying immunological "memory" can circulate which is unlikely in the light of the finding that recirculation occurs in the foetal lamb (Pearson et al 1976), which we are led to believe is immunologically virgin, but by the definition of the clonal selection theory must contain at least some antigen sensitive cells. However, in a study of materno-foetal responses to Mycobacterium leprae, it was found that a low molecular

weight substance released by lymphocytes crossed the placenta and sensitised the foetal lymphocytes (Barnetson et al 1976). Recirculation from blood to lymph, it would seem, is a physiological property of the cells themselves whether they be virgin or memory; undoubtedly cells can be stimulated to recirculate by antigen (Strober & Dilley 1973, Gery et al 1977), but initially the surface features of the cell must be of prime consequence.

Having discussed the need for a recirculatory system, what classes of lymphocyte have been found to traverse the "blood-lymph" barrier? It is assumed that if a cell is found in thoracic duct lymph, it is a recirculating cell. On the whole, this is true and it can be considered that most cells were probably in the blood no more than 18 hours previously. However, the input of cells which have divided in the lymph node and have been released, which cannot rightly be called recirculating cells, must be considered (Hall & Morris 1965). However, most of the studies on circulation have utilised radioactively labelled cells introduced into the blood, so we can be fairly certain that they are of the circulating pool if they appear in the thoracic duct lymph (TDL). The majority of these cells are of a T lineage (Sprent 1973) but up to 20% may be B cells depending on the animal studied. Using the "B" rat, produced by adult thymectomy, lethal irradiation and bone marrow cell reconstitution of rats, Howard and co-workers (1972a & 1972b) found that the thoracic duct lymph contains B lineage cells which are predominantly long-lived whereas the frequency of short-lived B cells is the same as in

normal lymph. These recirculating long-lived B cells take much longer to reach the thoracic duct lymph than normal TDL cells due to their taking a more tortuous route of movement through the lymph node. Thus, although the route of entry of both cell types is the same (Gutman & Weissman 1973, Nieuwenhuis & Ford 1976), T cells are more quickly mobilised on thoracic duct drainage than B cells. It has been suggested that immature B cells are short-lived and do not recirculate which is in some respects true in that B memory cells are able to recirculate (though probably not all do so) and short-lived cells do not contribute much to memory (Strober & Dilley 1973). However, it has been found that memory B cells, although recruited from the recirculating population in the secondary response are only recruited from the sessile B cell population in the primary response (Smith et al 1970). The argument from this experiment is that recirculation of B cells is not a memory phenomenon and hence is not antigen driven. That memory B cells are somewhat different is suggested by the observation that they can migrate to the thymus (Benner et al 1977) and that circulating B cells, accumulating in a stimulated lymph node are found in the T-dependent area (Gery et al 1977). From this mass of confusing data, it can be seen that T and B cells can be recirculating or sessile, virgin or memory, adding further support to the hypothesis that recirculation is a phenomenon of lymphoid cells and the tissues they migrate through rather than as a result of antigenic stimulation.

The recirculation of lymphoid cells, no matter of which class, essentially involves five stages:

1. The adhesion of lymphocytes in the blood to the high endothelial venules (HEV) of lymph nodes (and Peyer's patches).
2. The initiation of movement of the adherent lymphocytes through or between the HEV cells into the lymph node stroma.
3. The "sorting out" of the respective cell types within the lymph node into dependent areas.
4. The emigration of cells from the node into efferent lymph.
5. The circulation of cells through other lymphoid and non-lymphoid organs.

These stages require an "active" participation on behalf of the lymphocyte itself while the traffic in the blood and lymph is purely passive as a result of cardiac blood pressure. There is, however, a possibility that the different rates of fluid flow in blood vessels might result in a separation of cells on the basis of charge. This suggestion will not be considered here due to an almost complete lack of data on the subject.

Since the discovery that lymphoid cells recirculate, people have been trying to find out what control mechanisms might be involved. Up until recently, the majority of experiments have, intentionally or not, been involved with the first stage of recirculation, the adhesion of lymphocytes to HEV cells.

The first and probably the most repeated experiments on the control of recirculation were as a result of treating lymphocytes with various enzymes which affect the cell surface, followed by an examination

of their recirculation or localisation behaviour in vivo. The earliest hypothesis to explain the control of recirculation postulated specific glycoside units on the lymphocyte surface with corresponding receptors on the HEV cells (Gesner & Ginsburg 1964). In these experiments, Clostridium perfringens glycosidases were found to reduce the localisation of cells in the spleen and lymph nodes while increasing that in the liver, although more recent evidence disputes the latter fact (Ford et al 1976). Of the other enzymes used, trypsin has been the most popular. It has been found that trypsin treated lymphocytes do not localise at all well in the lymph nodes while their localisation in the white pulp of the spleen is almost unaffected (Woodruff & Gesner 1968). This effect decreases with time suggesting that the cell is able to resynthesise any units cleaved by the enzyme and that the mechanisms of spleen and lymph node localisations involve different recognition systems (Gesner et al 1969). Further experiments on the trypsin sensitivity of cells showed that sialylglycoproteins cleaved from the cell surface were rapidly (about 12 hours) resynthesised as determined by puromycin and temperature sensitivity (Woodruff 1974). However, it has been found that puromycin treatment of cells will also reduce their mutual adhesiveness and it is possible that this factor can alter the localisation patterns which were the sensitivity criteria in Woodruff's (1974) experiments (Curtis et al 1975a). This does not wholly argue for the concept of resynthesis of surface components but indicates that a number of factors must be involved in lymphocyte recirculation. Whether these proteins are the most important in the control of recirculation is not known since, although

their loss alters localisation patterns, they are by no means the only surface components removed by trypsin. There is now no doubt that trypsin does inhibit the entry of cells into lymph nodes from in vitro experiments (Ford et al 1976, Woodruff et al 1977), but the actual role of sialylglycoproteins in this mechanism is now to be doubted. In other early experiments, neuraminidase treated lymphocytes were found to localise in the liver and the lymph node entry was also seen to be lowered, although the liver sequestration was not considered to be the whole explanation (Woodruff & Gesner 1969). This increase was not due to a decrease in the viability of the cells (Berney & Gesner 1970, Wilg 1976) and a concomitant paucity of cells in the blood was thought to explain the decrease in lymph node entry (Freitas & de Sousa 1976a). This was confirmed and extended by Ford et al (1976), in an elegant study using an isolated, perfused mesenteric lymph node chain from the rat. Using this system, it was found that neuraminidase treated cells entered the lymph nodes at least as well as untreated cells, while the entry of trypsin treated cells was still inhibited. The explanation of the defective lymph node entry seen in the whole animal studies with neuraminidase in earlier years was that, due to the large accumulation of cells in the liver, the actual number of cells available in the blood for recirculation through the lymph nodes was decreased but the ability of those cells to enter the lymph nodes was not impared. Neuraminidase has been widely used in the study of cell surfaces of other cell types which may help us to understand what is happening in the lymphoid system. Neuraminidase, which cleaves cell surface

sialic acid from D-galactose residues may well be involved in the adhesion of cells by generating new binding sites for glycosyltransferases (Roseman 1970) or by reducing electrostatic forces of repulsion between cells (Lloyd & Cook 1974) and sialic acid also has consequences in the control of tumour metastasis (Gasic & Gasic 1962). There is some evidence to suggest that the hepatic localisation of cells may be controlled by the expression of sialic acid since neuraminidase treated red blood cells are cleared very rapidly by the liver (Berney & Gesner 1970) and spleen and are phagocytosed by Kuppfer cells and macrophages (Jancik et al 1978). A recent study of thymus lymphocytes, using the lectin peanut agglutinin (PNA), has come up with further support for this theory (Reisner et al 1976, Irlé et al 1978). It has been found that PNA, which binds specifically to D-galactose residues, is able to agglutinate whole thymus cells but not those thymus cells which are hydrocortisone resistant (HCR). These HCR thymocytes, as described earlier, make up 5-10% of the thymocyte population and have been found to express similar amounts of sialic acid and sialyltransferase activity to peripheral T and B cells while the whole thymus population shows little activity of either (Despont et al 1975), although Mehrishi & Zeiller (1974) and Sundaresan et al (1975) suggest that B lymphocytes have less sialic acid on their surfaces. Sialic acid expression may then be a maturational stage of T lymphocytes. Injected thymocytes will localise preferentially in the liver and it is suggested that the binding mechanism may be similar to that of the PNA molecule. Cells exhibiting large amounts of sialic acid would not be bound by the liver

according to this hypothesis, but unfortunately the conflicting evidence concerning cell surface sialic acid and the fact that B and T cells do not localise in the liver to the same extent (Wiig 1976, Freitas & de Sousa 1975) cannot be reconciled. Thus, the role of sialic acid in lymphocyte circulation may be important for the interactions with non-lymphoid organs but seems to have little bearing on the lymph node entry and hence recirculatory habits of cells. Trypsin, in comparison, is an enzyme of little specificity and the fact that it inhibits lymph node entry is hardly surprising considering the damage that would be inflicted to the cell surface. In addition, trypsin has been found to be a potent stimulator of DNA synthesis in B lymphocytes (Vischer 1974). Rannie et al (1977) have made the observation that the migration of lymphocytes into cell-mediated immune lesions as well as lymph nodes is trypsin-sensitive, whereas migration to the spleen and non-lymphoid organs is trypsin-resistant and indeed the localisation in these organs is often increased. Since neuraminidase decreases cell entry into the spleen (Freitas & de Sousa 1976a), it is possible that the trypsin-resistant effects above are sensitive to neuraminidase and that the two enzymes show no interreactions as regards recirculation control mechanisms. De Bono (1976), studying lymphocyte-endothelial interactions in vitro has found that there is a specific component of the endothelial cell surface which is neuraminidase labile and is responsible for lymphocyte adherence. However, this only concerns the adherence of lymphocytes to generalised endothelium and may be due to the removal of a receptor which will non-specifically

bind lymphocytes. That HEV cells are probably functionally distinct from generalised endothelium is evident from the work of Stamper & Woodruff (1976) on the binding of recirculating lymphocytes to lymph node sections. In this assay, recirculating lymphocytes bound very much more to HEV cells than to the surrounding endothelium.

Other workers have used neutral proteases (Wiig 1976) and have examined their effect on lymphocyte localisation. Protease treatment is found to decrease the lymph node localisation and increase the liver localisation. It was suggested that the proteases did not act by killing the cells since heat-killing the cells does not increase their localisation in the liver (not agreed with by Freitas & de Sousa 1976c). On the basis of the comparison of the effects of protease and neuraminidase from his work, Wiig proposed three types of receptor to be involved in lymphocyte circulation:-

1. For migration into Peyer's patches - resistant to protease and neuraminidase.
2. For lymph nodes - receptors sensitive to neuraminidase and protease.
3. For spleen migration - receptors mainly neuraminidase sensitive.

In the light of the more recent studies by Ford and co-workers using the isolated lymph node, this interpretation is now probably incorrect. In fact, due to the similarity of the structures involved,

the migration of cells into lymph nodes and Peyer's patches is probably controlled by the same mechanism, while migration into the spleen, which is via morphologically different structures, may be controlled by another mechanism.

At this point, it is necessary to point out that proteases are found naturally in serum (and probably lymph) and are essential for the functioning of many immune and non-immune systems. Specific proteases are of key importance in the activation of the complement system but trypsin, thrombin, plasmin and lysosomal proteases have also been found to affect the C3 component of complement (Götze 1975). Proteases are also essential for the correct interactions of glycoproteins in the cascade mechanism for blood coagulation giving rise to fibrin formation (Davie et al 1975). It is thus clear that we must view the actions of proteases in vitro on the in vivo localisation patterns of lymphocytes with care. The presence of serum proteases may have further, as yet unconsidered and undefined, effects on circulating lymphocytes.

Probably the most specific enzymes which have been used to reveal the complexities of the control mechanisms of lymphocyte circulation are the phospholipases A and C and sphingomyelinase C (Freitas & de Sousa 1976a,c). Phospholipase A (PL-A) has a fairly small chemical effect on the cell surface phospholipids, but a major effect on the distribution of labelled cells. The main defect seen is a decrease in lymph node localisation with transient alterations in spleen, lungs, liver and small intestine.

Phospholipase C (PL-C) has a far more drastic effect on phospholipids releasing from them a free phosphorylcholinegroup. Although the data provided by Freitas & de Sousa is incomplete compared to that of PL-A, PL-C causes a transient increase in the blood level and decrease in lymphoid organ levels but considering the detrimental effect of the enzyme on cell viability, this is not entirely surprising. This may be explained by the fact that the PL-C used was an impure preparation. Sphingomyelinase C (SM-C) is a specialised form of PL-C but was found to be too toxic for any cell traffic measurements to be made. The conclusions to this work are not clear; PL-A causes an accumulation of lysocompounds at the cell surface, known to decrease the mutual adhesiveness of cells (Curtis et al 1975a,b) and also mimics the effect of trypsin on cell localisation, although the possibility that the membrane conformational changes caused by trypsin (Nicolson 1972) and phospholipid alteration have the same mechanism has not yet been proved. Another observation is that PL-A_2 may be present in serum as the substance which causes a decrease in the adhesiveness of cells (Curtis & Greaves 1965, Curtis et al 1975a). It is then interesting to speculate that the adhesiveness of cells within an organ not directly permeated by serum might be greater than those in circulation which would help to maintain the integrity of that organ. PL-C and SM-C, which have more marked effects on phospholipids than PL-A, have such a detrimental effect on cell viability that any differences in localisation seen can probably be attributed to the general degradation of cellular function.

Another enzyme used has been hyaluronidase (Ford et al 1978) which cleaves the bond between D-glucuronate and 2-deoxy-2-acetamido-D-glucose in acid mucopolysaccharides. However, although this enzyme causes a marked leucocytosis, treatment of lymphocytes with it has very little effect on recirculation.

Although no definite enzymic effect can be attributed to the alterations in cell localisation so far described, it becomes abundantly clear that a considerable number of effects are responsible for regulating the interaction between lymphoid and other cells. Thus, sialic acid, phospholipids and constituents of the glycocalyx may all be relevant in circulation, each exerting their effects in different areas and on different receptors. The enzyme work has been of benefit in helping to elucidate the mechanism of recirculation but it has by no means answered all the questions.

As has already been mentioned, lectins such as PNA have the ability to bind specific cell surface sugars and effectively mask them from recognition and also to induce cell membrane changes. These abilities have also been utilised in the study of lymphocyte recirculation. Concanavalin A (Con A) has been shown to increase the localisation of labelled lymphocytes in the spleen at the expense of localisation in the lymph nodes (Gillette et al 1973). However, the defective lymph node entry is as a consequence of the increased level in the spleen since, in splenectomised hosts, treated cells localise equally well in the lymph nodes (Freitas & de Sousa 1975, 1976a). From the conclusions of the neuraminidase work, it would be interesting

to know the levels of cells in the blood in these experiments, but they have been curiously omitted. The fact that neither of the above two reports entirely agrees with that of Schlesinger & Israël (1974) indicates that the effect of Con A can be variable and that the incubation conditions in vitro are critical. Another lectin, phytohaemagglutinin (PHA), and bacterial lipopolysaccharide (LPS) were found to have similar effects to Con A (Freitas & de Sousa 1975, 1976b). Since lectins do not seem to affect the ability of a cell to enter the lymph nodes, this removes much of the support for the sugar receptor theory since the sugar would be masked. Con A appears to slow down the recirculation of T cells with the result that the localisation patterns with time of treated T cells are similar to that of untreated B cells (Freitas & de Sousa 1975). This suggests that the recirculation mechanisms for both cell types are similar and only the route and tempo differ. This alteration in recirculation rate could probably be caused by a cell membrane change as would be expected to be caused by the tetravalent molecule of Con A. Recently, Freitas et al (1978) have confirmed this suggestion by studying the effects of monomeric, dimeric and tetrameric Con A on lymphocyte traffic. Monomeric and dimeric Con A are unable to modify traffic which suggests that the alterations seen using tetrameric Con A are due to a cell membrane change, probably in the form of a change in fluidity which was suggested not to involve microtubles. These experiments lend more support to the suggestion made earlier that a combination of surface features in a particular configuration are necessary for normal recirculation.

In the past few years, there has been an increasing amount of effort put into the study of the role of sulphated polysaccharides of various types on lymphocyte recirculation. Gowans & Knight (1964), concerned about an earlier report of the possibility that heparin, a sulphated acid mucopolysaccharide, might cause the release of lymphocytes from lymph nodes into lymph (Jansen et al 1962), found that in the concentrations used in their experiments, it did not cause the release of lymphocytes into the lymph over and above normal levels. Later work (Bradfield & Born 1969) indicated that heparin causes a marked lymphocytosis without a measurable increase in the rate of supply of cells from the thoracic duct, which was due to an inhibition of the extravasation of cells from the blood. The ability of cells to enter the white pulp of the spleen was also compromised. In a further report (Bradfield & Born 1974), it was suggested that, in fact, heparin decreases the ability of cells to cross the blood-lymph barrier which implies blocking at the level of the high endothelial cell in the post capillary venules. An interesting observation from these experiments is that only acidic sulphated polysaccharides like dextran sulphate can cause the lymphocytosis seen as dextran phosphate, a similar negatively charged acidic phosphated polysaccharide, has no effect. Similarly, no lymphocytosis is produced by the positively charged diethylaminoethyl (DEAE) dextran or neutral dextrans. This is in some ways surprising since neutral dextrans can bind to all cells and increase their zeta potential, a measurement of surface charge (Brooks & Seaman 1973) and positively charged molecules would be expected to attach

to negatively charged cells by simple charge attraction. Negatively charged molecules can attach to negatively charged cells utilising Ca^{++} ions as cationic bridges (Dietrich et al 1977). Thus it seems that the role of surface charge in lymphocyte recirculation is difficult to correlate along with the fact that Con A, LPS and neuraminidase, which all alter the charge on cells, have no effect on recirculation through the lymph nodes (Ford et al 1978). One anomalous fact in Bradfield & Born's work (1974) is that chondoitin sulphate, an acid mucopolysaccharide, found in large amounts in the cell glycocalyx, unlike dextran sulphate, has no effect on the lymphoid system. However, Viklicky et al (1976) found that if blood glycosaminoglycans (strictly glycosaminoglycuranoglycans, another name for mucopolysaccharides) in the form of chondroitin sulphate are raised, then the localisation of injected labelled cells in the lymph nodes is reduced. Most of this work has been repeated by Freitas & de Sousa (1977) who also found that only sulphated polysaccharides cause a decrease in localisation of labelled cells in the lymph nodes with a concomitant increase in the blood. The effects were related to and differed slightly with the molecular weight of the dextran sulphate used, which was also found by Bradfield & Born (1974). They also found that neutral and positively charged DEAE-dextrans have no effect on lymphocyte localisation. Treatment of cells with dextran sulphate may also slow down their circulation through the marginal zone and red pulp of the spleen (Freitas & de Sousa 1977). In a further study on the effects of dextran sulphate, Ford et al (1978) set out to prove the hypothesis that sulphated proteoglycans (sulphated acid mucopolysaccharide-protein complexes as normally

found in vivo) may play a part in the selective migration of lymphocytes from the blood but without much success, which is hardly surprising when the hypothesis and the experimental conditions used are chemically different. Other results had suggested that there might be an acidic sulphated proteoglycan on the surface of HEV cells (a quite likely fact) which would be in competition with the dextran sulphate used in the experimental system. However, there was no evidence in favour of this idea and dextran sulphate was not found to bind to the surface of HEV cells in vivo. It has been found that the degree of sulphation of the polysaccharide is critical for the inhibitory effect on the lymph node entry (as well as the molecular weight of the dextran) and that HEV cells can possibly manufacture and release a sulphated molecule, a feature not exhibited by normal endothelial cells. Dextran sulphate has also been found to inhibit the adhesion of cells in tissue culture which may reflect a general effect on the adhesion of all cells explaining the decreased lymphocyte-endothelial interaction seen in these experiments (Bremerskov 1973). Another effect of dextran sulphate is to stimulate DNA synthesis, but only in B lymphocytes, while neutral dextrans have no effect. It probably acts by entering the cell (which it does very rapidly) and altering some internal control mechanism (Diamanstein et al 1973). An interesting point has been made by Dietrich et al (1977) concerning sulphated mucopolysaccharides (SMPs) like chondroitin sulphate A/C, chondroitin sulphate B and heparitin sulphate. These are present on the cells of all tissue-organised life forms and show structural and type differences depending on the tissue or organism

of origin. Thus, differences in SMP expression meet most, if not all, the requirements for a role in the process of specificity for recognition and adhesion between tissues. Although the picture is still very clouded, it seems plausible that the hypothesis of Ford et al (1978) could be correct - a sulphated proteoglycan on the surface of the high endothelial cells which could provide the specificity for the recognition and adhesion stages of lymphocyte recirculation.

There are also a number of other effectors altering lymphocyte recirculation - substances, the site of action of which is not known, but which alter recirculation rates. They may or may not affect the initial stage of lymphocyte-HEV cell recognition and adhesion. Anaesthesia, injection of fluid, prednisolone and corticotropin have all been found to reduce recirculation in rats (Spry 1972) presumably by some steroid action which may only affect circulating T cells. T lymphocyte recirculation has also been found to be slowed by the activation of C3 complement by cobra venom factor although this effect may be due to an alteration of the macrophages lying along the T lymphocyte traffic route which may increase the rate and avidity of lymphocyte-macrophage interaction (Spry et al 1977). The chemical state of end groups of components of the cell surface appears to be important in that lymphocytes oxidised with sodium periodate in vitro do not localise in the lymph nodes, an effect which can be abolished by the subsequent in vitro reduction with sodium borohydride. Reduction alone has little effect (Zatz et al 1972b). The use of radioactive sodium chromate, also an oxidising agent, to label cells

for injection in vivo appears not to alter the cells' localisation patterns (see results). The blood flow to lymph nodes must also affect the entry of lymphocytes into nodes. If the volume of blood reaching the node is increased, then there is an increased number of lymphocytes available to enter the node. Similarly, if the speed of flow increases, then haemodynamic factors will make it more difficult for an adherent lymphocyte to remain attached to an HEV cell prior to migration into the node. It has been found that antigen stimulation increases the blood flow by as much as four times to a regional node and that there is an increase in lymphocyte traffic as a direct result of this increase in flow (Hay & Hobbs 1977). However, since only one lymphocyte in ten (less by other estimates) entering a normal node by the venous route, migrates out of the blood, then an increase in traffic could be as a result of a greater number of cells migrating out of the blood for the same original rate of blood flow to the node (Hall & Morris 1965). This would assume that the HEV cell sites for lymphocytes are not "saturated" in the normal condition.

Sodium azide treatment of lymphocytes has also been used (Ford et al 1978). This substance, a metabolic inhibitor, stops cell surface components capping and reduces the entry of injected cells into the lymph nodes. Whether this reduction is due to the inability of the cell surface components to cap or whether as a result of other metabolic blocks is not known. However, azide does not inhibit the maintenance of surface villi (Loor & Hägg 1975), as mentioned earlier, which have been considered necessary for the initial recognition of endothelial cells (Anderson & Anderson 1976).

Anderson & Anderson (1976) found that regional perfusion of the rat mesenteric lymph node with EDTA (ethylenediaminetetraacetic acid, a divalent cation chelator) causes a reduction in the adherence of blood lymphocytes to HEV cells. Divalent cations are normally required for adhesion between cells and the case of adhesion between lymphocyte and endothelial cells is probably no exception. Whether or not EDTA acts on this mechanism alone and no other cellular process is not clear.

The major histocompatibility complex and its products also seem to have some role in recirculation, again possibly affecting the adherence to high endothelial cells. Zatz et al (1972a) found that if there is a strong H-2 histoincompatibility between injected cells and the recipient, then cell localisation is impaired. Earlier experiments (Bainbridge et al 1966) had suggested that allogeneic cells recirculated as well as syngeneic cells. That histocompatibility differences might be recognised at the lymphocyte-endothelial adhesion stage is evident from the observation that only autologous cells can recirculate from blood to lymph while even allogeneic cells can circulate from afferent to efferent lymph (Frost et al 1975). There is at least another explanation of this result. Histoincompatible lymphocytes might be able to cross the HEV cell barrier but would be immediately recognised as foreign by resident macrophages within the lymph node parenchyma and thus be destroyed. The route of migration from afferent to efferent lymph does not require movement through macrophage-infested areas and so incompatible cells might escape recognition.

The supernatants from cultures of Bordetella pertussis, which cause a leucocytosis, decrease the ability of lymphocytes to enter the lymph nodes in the same manner as sulphated polysaccharides. Pertussis treated lymphocytes enter the lymph nodes of normal and pertussis treated mice less than controls and the spleen level is not quite as high (Morse & Barron 1970). The treated cells are not sequestered in any other organ and remain in the blood (Taub et al 1972) so the stage which must be blocked is the initial lymph node entry. Pertussis factor has now been isolated and is a four polypeptide subunit, lipid and carbohydrate free protein of 74,000 daltons molecular weight (Morse & Morse 1976). Whether this substance acts on the lymphocyte or HEV is not clear, although it is suggested that it does in fact act on the HEV.

The adhesive state of the cells so far mentioned concerns the adhesion to endothelial cells. It is possible that the adhesion between like lymphoid cells can be used as a guide to the adhesive state of that cell with respect to different cell types. Thymocytes having different mutual adhesivenesses have been shown to have different localisation/recirculation patterns (Evans & Davies 1977) but it is not certain whether cell maturation has anything to do with this system. As mentioned earlier, puromycin, which reduces cell adhesion, also alters cell localisation but in this case the other effects of puromycin probably have some bearing on the result. In a preliminary experiment, it was suggested that a substance released from T lymphocytes in culture which decreases the mutual adhesiveness of B lymphocytes can alter the recirculation of lymphocytes in

nude mice (Curtis & de Sousa 1975). Results in this thesis will demonstrate further effects of these substances.

It has been known for some time that iron and iron-containing compounds are involved in Hodgkin's disease, a cutaneous T cell lymphoma (Beamish et al 1972). Recently, it has been suggested that iron compounds and iron binding proteins might also modify lymphocyte circulation (de Sousa et al 1978). Lactoferrin and transferrin, two iron binding proteins have been found to bind to lymphocytes (van Snick & Masson 1976, Phillips 1976) and lymphocytes bearing surface ferritin have been found in patients with Hodgkin's disease and breast cancer (Moro et al 1977). Moreover, large amounts of ferritin have been found in the spleens of patients with Hodgkin's disease (Bieber & Bieber 1973) and circulating T lymphocytes are specifically sequestered by the spleen (de Sousa et al 1977). Iron salts have also been found to reduce the incidence of rosettes of sheep red blood cells and human T lymphocytes (de Sousa & Nishiya 1978) although the exact relevance of this observation is hard to imagine. de Sousa (1978) has thus suggested that lymphocytes might have receptors for iron-binding proteins or metal-protein complexes and would therefore be able to migrate to areas rich in metal-proteins. Pretreatment of cells with iron salts might be expected to block such receptors and divert cells to other sites. This hypothesis was apparently experimentally proved although there is some controversy concerning the viability of cells following treatment with iron salts. The role of iron in lymphocyte positioning is still a moot point complicated by interpretations of observations on pathological states

being extrapolated to normal conditions. At the present time, it would be incorrect to say that the actions of iron salts or iron-protein complexes have been proved to be a major control mechanism in lymphocyte circulation.

At this point, it is worth mentioning further some recent experiments by Woodruff and her colleagues which have already been cited. The system involves an in vitro model of lymphocyte recirculation by measuring the adhesion of circulating lymphocytes to lymph node sections. When a lymphocyte suspension is layered over a fixed section of a syngeneic lymph node, cells stick preferentially to the HEV cells but not to other vascular structures (Stamper & Woodruff 1976). Adherence to HEV cells is a property of recirculating cells and thymus and bone marrow cells only adhere poorly. The strange features of the model are:

1. Maximal adherence of cells occurs at 7°C, and is greatly decreased at 1°, 24° and 37°C. This is explained by the increased stability of an enzyme complex at 7° although there is no evidence given for this.
2. The HEV cells must be fixed with glutaraldehyde for optimal adherence though there is some suggestion that it does work without fixation. It must also be borne in mind that the cells are adhering to sections of cells so cytoplasmic effects may come into play.
3. The lymph nodes used must be in perfect physiological condition, i.e. from non-stressed, non-infected rats. Even then, there were times when the adherence did not

occur which could not be explained.

(Stamper & Woodruff 1977)

Despite these incongruities, the model is interesting as it is possible to study directly lymphocyte-endothelial interactions without the influence of many of the other factors that occur in vivo. It has been found that adherence is energy dependent, sensitive to calcium ions and the calcium ionophore A23187, involves trypsin-sensitive surface determinants, does not involve surface sialic acids and there is no evidence of microtubule involvement. However, the detrimental effect of cytochalasin B on adherence suggests that microfilaments may be involved (Woodruff et al 1977). Since the HEV cells are fixed and metabolically inert, adherence is a feature of the lymphocyte surface so that glutaraldehyde fixed lymphocytes do not adhere. Whether the use of the model is valid with respect to the actual mechanisms involved in vivo has not yet been evaluated, but it certainly seems to confirm many of the in vivo experiments previously performed. The only stage it can examine is the initial adherence and recognition, about which we already know the most.

In conclusion, despite the number of experiments performed, little is known about the first stage of recirculation. The mechanism appears to be fairly complex requiring the integrity of a number of cell surface components and probably some active role by the HEV cells. Once the lymphocyte has adhered to the HEV cell, it must migrate into the node - the second stage of recirculation.

Even less is known about this second stage, indeed most of the reports

so far published have been concerned with electron microscope studies, the earliest of which is again the work of Gowans (Marchesi & Gowans 1964) who suggested that migrating lymphocytes actually penetrate the cytoplasm of high endothelial cells, entering on the luminal side and leaving in the basal region. They also noted that only small lymphocytes are able to cross the HEV cell barrier and not neutrophils or large lymphocytes. Other workers have agreed with both these observations but in a study of some 800 electron microscope pictures of lymphocytes traversing HEV cell borders, Schoefl (1972) found that 91% of the lymphocytes examined were clearly intercellular and 9% were surrounded by endothelial cytoplasm, disputing the earlier work of Marchesi & Gowans. Schoefl's work has subsequently been confirmed (Wenk et al 1974, Anderson & Anderson 1976) and the explanation of the apparently intracellular cells has been that:

a. Too few sections were examined by Marchesi & Gowans.
b. Those cells appeared intracellular due to the orientation of the sections as serial sections were not examined.

It is now generally acknowledged that lymphocytes enter the node by migrating between adjacent HEV cells caused by a temporary reduction in lateral adhesion allowing through movement of the cell. The tall lumino-basal height of the HEV cells may allow sustained and constant cell traffic without causing excessive fluid loss into the node (Schoefl 1972). The most painstaking electron microscope and functional studies on the migration of cells between HEV cells have been made

by the Andersons. In a very thorough examination of the structure of the microvasculature of lymph nodes (Anderson & Anderson 1975), it has been found that distal venous sphincters, innervated by unmyelinated nerve fibres, permit the regional control of blood flow through the venules. Thus, a substance like adrenalin will cause a constriction of the venous sphincters and hence a reduction in blood flow to the node. It is on this control mechanism that the effects of antigenic stimulation on blood flow must occur (Hay & Hobbs 1977). It has also been suggested that when the blood flows from the capillaries into the venules, the sudden increase in vessel volume would slow the blood flow and aid the attachment of cells (Anderson & Anderson 1976) although, since the total volume of the capillary beds is greater than in the draining veins, the blood flow might, in fact, increase. In this study, it was found that lymphocytes attached to HEV cells through contact points, which were able to resist shear forces sufficient to flush away other blood elements. These contact points look very similar to the microvilli visible in the SEM studies of van Ewijk et al (1975).

The high endothelial venules of lymph nodes and Peyer's patches have been found to have a similar complex structure (Anderson et al 1976). The plump endothelial cells are ensheathed by a series of overlapping reticular plates and connective tissue so that any migrating lymphocyte has to move around the edges of the plates to enter the node, a satisfactory way of minimising vascular leakage also aided by the dimensions of the HEV cells themselves (Anderson et al 1976). Although there was no evidence to believe so, Anderson & Anderson

(1976) suggested, probably correctly, that, after attachment of the lymphocyte to the HEV cell, the migration into the node is directed by a chemotactic gradient. Although another mechanism might be involved, this may be a feasible explanation for the direction of movement of cells into the node which may not however be exclusively one-way as discussed below.

The work of Gowans & Knight (1964) suggested that the major route for recirculation of lymphocytes is from blood to lymph and Anderson & Anderson (1976) agreed that the traffic through the node is probably unidirectional from blood to lymph in a continuous or intermittent movement. They also noted that migrating lymphocytes showed a cytoplasmic polarity oriented in the direction of movement and that 92% of migrating cells were moving into the node from the venule. The actual study of lymphocyte locomotion in vitro, other than that of the chemotactic reactions of lymphocytes (e.g. Ward et al 1971, 1977), has been a subject curiously neglected in the interpretation of lymphocyte traffic. Rydgren et al (1976a) have studied the locomotor responses of human lymphocytes in autologous plasma clots by time lapse cinemicrophotography and have found that the locomotion of normal lymphocytes, from the examination of "hundreds and thousands of cells", is extremely rare. However, after membrane stimulation with PHA, or antiimmunoglobulin, macrophages or isologous lymphocytes, also all found to stimulate the chemotaxis of lymphocytes (Ward et al 1977), lymphocytes are induced to move, or at least to adopt the characteristic shape polarity of motile cells. They concluded that the basic polarity of the moving lymphocytes

provided a basis for the analysis of lymphocyte traffic within the node. Utilising this observation in a further study (Rydgren et al 1976b), it was found that, analysing the movement of lymphocytes (with the characteristic shape) relative to the basement membrane of the venules, 69% of the 118 lymphocytes examined were apparently on their way into the venule from the lymph node parenchyma, in contradiction to the more definite results of Anderson & Anderson (1976). This suggested that lymphocyte traffic might be bi-directional with the major route being from the lymph node into the venule which conflicts with the current dogma and agrees to some extent with the work of Sainte-Marie and co-workers.

It was found that there were more lymphocytes in the blood leaving the node than arriving at it (Sainte-Marie et al 1967) which suggested that lymphocytes entered the blood from the node. Further experiments showed that blood borne labelled lymphocytes in the dog (Sainte-Marie et al 1975) and intramediastinally injected thymocytes in rats (Sainte-Marie & Peng 1975a) arrived at lymph nodes via the afferent lymphatics and the subcapsular sinuses. An interesting, although not particularly relevant point to this discussion from these experiments was the difference in localisation of medullary and cortical thymocytes injected intramediastinally. The medullary thymocytes formed pseudo-follicles while the cortical thymocytes migrated into the extra-follicular zone of the cortex (Sainte-Marie & Peng 1975b).

From all these experiments, it is apparent that the direction of

lymphocyte flow might be bi-directional, although this would further complicate any of the postulated mechanisms. Why should some cells migrate into the blood and what are their characteristics? The experiments just described do naturally have their faults. Rydgren et al (1976b) only studied 118 migrating lymphocytes while Anderson & Anderson (1976) examined 146 motile lymphocytes, although only 136 are shown in their scattergram. Analysing the data of Rydgren et al, it is interesting to note that of these 118 cells examined, 67 were from normal animals, 40 from animals treated with erythrocytes, 6 from ferritin treated and 5 from typhus vaccine treated animals, i.e. those undergoing an immune response. From the scant data on control animals, 73% of the lymphocytes studied were moving into the venule from the lymph node parenchyma. The photographs published are not particularly clear and the decisions of the direction of movement of some of the cells must have been very subjective. Some of the work of Sainte-Marie also has its faults - intramediastinally injected thymocytes might be expected to arrive in the nodes by the afferent lymphatics through the lymph channels draining the mediastinal cavity, although few cells seem to arrive via the afferent lymphatics to the node (Hall & Morris 1965). Another problem is that cells have been observed migrating from the lymph node parenchyma into the venule but once in the venule lumen, will a lymphocyte re-migrate back into the node by the classical route or will it be carried away into the blood stream? Since the lymphocytes migrating into the venule are capable of moving, then their membranes must be activated in some way as interpreted by

Rydgren et al (1976a). Once in the lumen of the venule, is it possible for these cells to readhere to HEV cells or can only cells with non-activated membranes adhere to the HEV cells in the first place? Which comes first, adhesion or membrane activation? This theory is difficult to reconcile since the localisation of labelled intravenously injected lymphocytes in the lymph nodes within a few minutes is rather too rapid to suggest a route via the afferent lymphatics, but traffic experiments up until now have only really considered the localisation of cells after intravenous injection which may be bypassing some key step.

The direction and stimulation of movement into the node is still an area of great interest and of extreme difficulty to study in that it is impossible to cannulate all the routes into and out of a single node without disrupting normal physiological integrity. The system of the isolated perfused node as described by Sedgley & Ford (1976) is a useful beginning but only the venous system was able to be cannulated. Their experiments showed that migration into the node was strictly one way but in the light of other conflicting reports, it is not correct to completely exclude the possibility of some bi-directional movement.

The initial adhesion to HEV cells has often been described as a case of "specific adhesion" only involving lymphocytes and no other leucocyte types. However, this is not necessarily so since other cell types might adhere to the HEV cells but be unable to migrate into the node. In this case, it could be said that the migration stage,

or at least the initiation of migration, is specific for lymphocytes since a signal might only stimulate lymphocytes to move and other cell types would subsequently become detached by the flow of blood. In support of this concept, de Bono (1976) suggests that pretreatment of lymphocytes with heparin and trypsin, which have already been described as reducing recirculation, does not prevent their initial adhesion to endothelium but rather the migration through the endothelial layer itself, although Woodruff et al (1977) have found that trypsin inhibits adhesion to HEV sections in vivo. Another point about lymphocyte movement concerns the observation by Rydgren et al (1976a) that normal blood lymphocytes are not motile. Since the migration into and through the node requires movement, then the cell must be stimulated in some way but sometime between the entry into the lymph and return to the lumen of an HE venule, the lymphocyte must lose its ability to move, and require restimulation to enter the node again.

After a short while, the recirculating lymphocyte has crossed the endothelial cell barrier for the third stage of recirculation. The stage of migration through the HEV barrier takes only a few minutes in comparison to the hours taken for the cells to "sort out" into B and T areas within the node. The first two stages of lymphocyte recirculation that have been described are probably not specific to B or T cells since they both arrive in the node by the same route (Gutman & Weissman 1973, Nieuwenhuis & Ford 1976). The cells, having arrived in the node, remain fairly immobile in this region for a few hours during which time cellular co-operation for the

initiation of the immune response is thought to occur (Nieuwenhuis & Ford 1976). This area, in the deep zone of the lymph node cortex, is designated a T-dependent area. If homogeneous populations (or at least of the same lineage) are radioactively labelled and injected intravenously, after 24 hours these cells will be found localised in specific areas of the lymphoid organs. It is particularly this process of migration to dependent areas that has been called ecotaxis by de Sousa (1971). T cells will migrate preferentially to the mid and deep cortex of lymph nodes, the inter-nodular spaces of Peyer's patches and the periarteriolar lymphatic sheath of the white pulp of the spleen (Parrott & de Sousa 1971, Mitchell 1972). The nodules and medulla of lymph nodes, plasma cells in Peyer's patches and the peripheral areas of the white pulp and red pulp of the spleen are known as T-independent or B-dependent areas.

After six hours or so, B and T cells have segregated into their respective dependent areas where they remain for a further period of up to 36 hours prior to emigrating from the node. The processes that control the activities of the lymphocytes from the stage of segregation to entering the efferent lymphatics some time later is a complete mystery, mainly due to the lack of work in this field area result of practical difficulties. At this point in time, no mechanism for the processes of sorting out and emigration have been postulated but in the discussion to this thesis a possible mechanism involving interaction modulation factors (IMFs) is suggested. These IMFs are released by homogeneous populations of lymphoid cells and act on cells of the opposite type by reducing their mutual adhesiveness -

i.e. T cell IMF affects only B cells and vice versa (Curtis & de Sousa 1973, 1975). However, there are certain features of the cells within lymph nodes that may help us to understand this process further - T cells have been found to alter the interactions between B and T cells possibly by the action of an IMF (de Sousa & Haston 1976).

Lymph nodes contain not only lymphoid cells but also a large number of structural cells and macrophages. These macrophages are known to line the T lymphocyte traffic routes in the lymph node (Spry et al 1977) and may be able to exert some modulatory effect on passing cells. Indeed, Scollay et al (1976) have suggested that macrophages in nodes "groom" cells on their passage through, which removes detritus collected during circulation in the blood and makes them more immunologically active. It has also been suggested that the process of lymphocyte trapping which occurs when the lymph node is stimulated by antigen may also be initiated by the action of macrophages (Frost & Lance 1974). Thus, it is possible that the interaction with other cell types in the node might cause migration to occur.

It was stated earlier that the passage of cells through the lymph node is a process that is independent of the major histocompatibility complex (Frost et al 1975), which hints at a more overall cellular control mechanism that can be explained by the interaction modulation factor theory rather than a specific recognition process, as might occur with the adhesion to HEV cells. A strange feature is revealed in the report of Frost et al (1975) - the time for recirculation between blood and lymph in sheep is given as 27-36 hours while the time for

circulation from afferent to efferent lymph is only 6-12 hours (recirculation times from blood to lymph in the sheep are longer than in the rat and mouse). Since cells have been seen to cross the HEV cell barrier into the lymph node in 15 minutes or less (e.g. Gowans & Knight 1964), then there is a period of at least 15 hours unaccounted for. Cells from the afferent lymphatics arrive at the node in the region of the sub-capsular sinuses, a B dependent area, and the difference in time might be explained by an accelerated movement through the node of cells arriving by this route. Alternatively, examination of lymph node structure shows that the route of migration between blood and efferent lymph requires movement through dense areas of resident lymphocytes and macrophages, while that between afferent and efferent lymph only requires movement along large lymph channels, altogether a less tortuous pathway. The proportion of B cells in the two different populations is important also since B lymphocytes take two to three times as long as T lymphocytes to recirculate from blood to lymph (Nieuwenhuis & Ford 1976). Consideration of these observations may help in formulating a theory of how cells sort out within the lymph node.

The fourth stage of recirculation is the emigration of cells from the node into efferent lymph channels and back into the blood. How this occurs is not known at all. Perhaps after a period of sojourn in the node, the cell's adhesiveness to its partners is reduced and it meanders throughout the nodal labyrinth until it reaches the lymph channel. At this time any explanation is purely speculative and will probably be an integral part of the explanation of sorting-out

within the node.

Up until now, this account has been concerned with the recirculation of lymphocytes through lymph nodes, but the spleen and non-lymphoid organs play a predominant role in the fifth stage of lymphocyte recirculation. In experiments on the isolated perfused pig spleen, it is found that the daily output of small lymphocytes from the thoracic duct is insufficient to account for the mean transit time of small lymphocytes through the blood compartment. It is likely in this case that release of lymphocytes from the spleen makes up the deficit (Pabst & Trepel 1975), although following perfusion of an isolated rat spleen it is found that the rate of release does not exceed the rate of entry until at least 6 hours after the introduction of cells (Ford 1969). Another fact that indicates the necessity of the spleen in recirculation is that the rate of recirculation in pigs is four times faster in a normal animal than in a splenectomised animal (Pabst & Trepel 1976). The mechanism of entry of cells into the spleen is very different to that into the nodes. No high endothelium is seen and the entry is not so selective. The transit times for cells in the spleen is also different; the maximum traffic time of cells being about 6 hours as opposed to up to 28 hours in lymph nodes (Ford 1969). How the spleen modifies recirculation is still a mystery, although the findings of Pabst & Trepel (1976) on the speeding up of recirculation by the spleen may not be directly applicable to other species since the recirculatory system of the pig is a unique case (Binns 1973). A further point that must be considered is the production of cells by the spleen, which has been estimated as being in the region of 4.6×10^9 lymphocytes per day in the pig (Pabst et al 1977), which can only be short

lived cells such that normal levels of lymphocytes are maintained. Other lymphoid and non-lymphoid organs, like the bone marrow, liver and lungs, doubtless have some role in controlling recirculation and this point will be discussed further in the latter part of this thesis.

From this account, it is fairly obvious that experiments on the control of lymphocyte recirculation have been centred around the interactions of lymphocytes with endothelial cells in the lymph node. Although this stage is undoubtedly of prime importance, the two final stages of recirculation that have been mentioned are often neglected. In addition, it is not only the interaction of lymphocytes with endothelial cells that controls recirculation but also their interaction with many other cell types, not least other lymphocytes.

The work presented in this thesis examines two aspects of lymphocyte circulation:

1. The role of the adhesive state of the lymphocyte surface.
2. The interactions between circulating lymphocytes as a control mechanism.

The results show that measurements of the adhesive state of the cell in vitro are not necessarily a good guide to their localisation patterns in vivo and that lymphocyte-lymphocyte interactions, which can occur in lymphoid and non-lymphoid organs, are probably of key importance in maintaining lymphocyte circulation on a gross scale.

CHAPTER FOUR

MATERIALS & METHODS

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4.1 Media

HEPES buffered Hanks plus Medium 199 (HH199).

90ml 10mM HEPES (Sigma; N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid) in distilled water pH 7.4.

5ml Hanks' 10x concentrate (Flow Labs.).

5ml Medium 199 10x concentrate (Gibco Biocult).

pH adjusted to 7.4 with 2N NaOH.

RPMI 1640

90ml HEPES water (as above).

10ml RPMI 1640 10x concentrate (Gibco Biocult).

1ml 4.4% sodium bicarbonate solution (BDH).

pH adjusted to 7.4 with 10N NaOH.

During the course of this work, problems were encountered with a batch of Medium 199 as a result of the medium being toxic (acknowledged by Gibco Biocult Ltd.). For this reason, a change to RPMI was made even though subsequent batches of 199 were found to be non-toxic. The adhesiveness of cells in the two media differed slightly (A. S. G. Curtis, personal communication) and thus no experiments using HH199 and RPMI are directly compared. A correction factor for use with measurements in RPMI medium was calculated and applied so that all adhesion values are expressed with respect to a standard HH199 medium.

The osmolarity of HH199 was 292 mOsmol/kg and RPMI 280 mOsmol/kg compared to 285 mOsmol/kg for normal mouse serum as measured by a Knauer freezing-point depression osmometer.

Hanks-HEPES. (HH)

90ml HEPES water.

10ml Hanks' 10x concentrate.

HEPES buffered salt solution (HSS).

NaCl	9g
KCl	0.4g
$CaCl_2.2H_2O$	0.076g
$MgCl_2.6H_2O$	0.1g

Made up to 1000ml with 10mM HEPES water, pH 7.4.

Foetal calf serum. (FCS).

When used, foetal calf serum (Gibco Biocult) was heat inactivated for 45 minutes at 56°C and used at a concentration of 10% in complete media.

Ammonium chloride solution.

0.83% ammonium chloride (BDH) in 10mM HEPES water, pH 7.4.

Ficoll-Hypaque.

Stock solutions of 9% and 14% Ficoll 400 (Pharmacia) and 33.9% Hypaque (sodium diatrizoate, Winthrop) were made up in distilled water and autoclaved. The mixtures used experimentally varied depending on the cells to be separated and are described in the appropriate sections.

Gelatin.

2.5% gelatin (BDH) was dissolved in 0.9% saline.

White blood cell counting diluent.

2% acetic acid in distilled water plus a small amount of crystal violet.

Formol-saline.

90ml 0.9% saline.

10ml 40% formaldehyde.

Trypan blue.

0.2% trypan blue (BDH) in HH.

Glutaraldehyde.

TAAB Ultrastructure grade glutaraldehyde (25%) diluted to 1% with RPMI before use.

Chemicals.

All chemicals used were 'Analar' grade where possible and water was double glass distilled.

4.2 Drugs & Antisera.

Cyclophosphamide (CPP; Endoxana, WB Pharmaceuticals Ltd.). Stock solution at 20mg/ml diluted with saline for injection intraperitoneally at a level of 200µg per gram body weight.

Anti-lymphocyte serum. (ALS, Searle Diagnostics, Batches 13 & 14). Injected intravenously or subcutaneously at a predetermined dose.

Anti-mouse immunoglobulin serum (Anti-Ig, Nordic Immunologicals and Gibco Biocult). Raised against isotypic mouse immunoglobulins.

Anti-Thy 1.2 serum (Searle Diagnostics). Raised in mice exhibiting only Thy 1.1 antigen against Thy 1.2 to give an allogeneic antibody with little non-specific cross-reaction.

4.3 Animals

CBA/ca female mice from 6-16 weeks old were used throughout. They were obtained from the following sources:

Department of Bacteriology & Immunology, University of Glasgow

ARC IRAD, Compton, Berkshire

Bantin & Kingman, Hull

OLAC, Bicester

Animals were housed in the Institute of Virology, University of Glasgow.

4.4 Animal Handling Techniques

Mice were kept ten to a cage with an area of 450cm^2 on peat and hay bedding with food and water ad lib. Following delivery from the breeders, mice were allowed to recover for at least two days before being used in any experiment.

The CBA/ca mouse is thought to be a genetically pure strain identical throughout the world but it was obvious that mice from different sources showed features that were consistently different, e.g. lymph node size. For this reason, it was not valid to compare results between different sources of mice and separate experiments were always done on mice from the same batch and of the same age.

Injection methods.

1. Subcutaneous. Volumes up to 0.5ml were delivered in a posterior direction in the skin fold in the scruff of the neck.
2. Intraperitoneal. Volumes up to 0.5ml were given into the lateral peritoneal areas away from the mid-line, care being taken not to enter or damage the viscera.
3. Intravenous. Following warming in an electrically heated cage, volumes up to 0.25ml were given slowly into the lateral caudal vein.

Exsanguination. Mice were anaesthetised using anaesthetic grade diethyl ether and exsanguinated by rupture of the aorta. Blood was collected with a needle-less syringe.

All experiments were conducted within the restrictions of the 1876 Cruelty to Animals Act covered by the licence to experiment on living animals and certificates A and B.

4.5 Glassware

All glassware was baked in an oven at 160°C before use.

Siliconising - glassware was treated for 10 seconds with 1% silicone fluid (MS 1107) in ethyl acetate followed by six washes in distilled water and baking at 160°C for one hour.

Subbing - this treatment was used to coat microscope slides prior to use for autoradiography to ensure good adhesion of the emulsion. Slides were dipped in a solution containing 5g gelatin and 0.5g chrome alum in one litre of distilled water, allowed to drain and dried in a dust-free atmosphere.

4.6 Preparation of cell suspensions

1. Normal cell suspensions.

Lymph nodes (inguinal, axillary, brachial and mesenteric) were removed and gently teased apart in medium using a scalpel. The tissue clumps were then aspirated through a needleless syringe to release the majority of lymphoid cells from the reticular network. The suspension was then filtered through a small plug of glass wool which had been prewashed with medium in the bottom of a syringe; this treatment removed all cell clumps and non-lymphoid tissue and results in a preparation with a viability in excess of 90% as measured by the exclusion of 0.2% trypan blue or nigrosine. The same method was later used to prepare thymus and spleen cell preparations although the latter is a heterogeneous population which required further purification.

2. B cell enriched suspensions.

(a) Using ALS. Following three daily intravenous or subcutaneous injections of ALS at a suitable, previously determined dose, lymph nodes were removed and prepared as above. Usually one additional passage of cells through cotton wool was necessary to remove dead cells. ALS kills all cells in circulation and is thought not to enter the lymph nodes. Since the majority of T cells recirculate, then the B cell component found in lymph nodes will be enriched with respect to T cells. The preparations used were at least 90% surface immunoglobulin positive as measured by fluorescent anti-IgG.

(b) Using anti-Thy 1.2 serum. Lymph node cells were treated in vitro with anti-Thy 1.2 serum at an appropriate titre for 30 minutes

at 2°C and then with complement for 30 minutes at 37°C. This causes the lysis of all cells exhibiting surface Thy 1.2 antigen (i.e. T cells). Dead cells were removed by passage over cotton wool. This technique was rarely used due to a low cell yield.

3. T cell enriched suspensions.

(a) Nylon wool columns. Lymph node cells were filtered through nylon wool as described in detail by Evans & Davies (1977) from a technique introduced by Julius et al (1973) and Greaves & Brown (1974). The resulting population was greater than 90% Thy 1.2 positive as measured by a fluorescent antiserum. The same method was used to fractionate thymus cells resulting in a subpopulation with increased adhesiveness.

(b) Using anti-Ig. Lymph node cells were treated in vitro with anti-Ig serum and complement as in 2(b). The resulting population was greater than 90% Thy 1.2 positive (this technique suffered the same problems as 2(b)).

(c) Cyclophosphamide treatment. Lymph node cells were removed three days after one single intraperitoneal injection of CPP (Turk & Poulter 1972). This substance kills all dividing cells and, since the majority of B cells are short lived, then T cells are relatively enriched.

4. Purification of spleen cells. Spleen cell preparations were well washed, resuspended in 4ml of medium and layered on to a mixture of 4.2ml 9% Ficoll plus 2ml 33.9% Hypaque in a siliconised 10 x 1.5cm test tube (modified from Bøyum 1968). The tube was spun at 400g. for 30 minutes at 18-20°C following a quick acceleration

to working speed. The band at the medium/separating mixture interface, of splenic lymphocytes (splenocytes), was then removed and washed free of Ficoll/Hypaque. The separating mixture has no effect on the localisation ability of cells (W. L. Ford, personal communication). This same technique was used to purify lymphocytes from spleens of mice treated with ALS. The resultant suspensions contained greater than 90% B cells.

5. Preparation of leucocyte rich plasma. Heparinised blood was added to twice its volume of 2.5% gelatin, mixed well and allowed to stand at 37°C for 60 minutes. The resulting cellular supernatant was then taken and washed well with HH medium and resuspended at about 10^6 cells/ml prior to being spun on to a slide in a cytocentrifuge.

6. Preparation of blood lymphocytes. Heparinised blood was diluted 1:2 with HH. 0.5ml mixture was layered on top of 1ml of Ficoll/Hypaque (21 parts 9% Ficoll:10 parts 33.9% Hypaque) in a siliconised 6 x 1cm glass tube, spun at 400g. for 20 minutes at 20°C, the medium/separating mixture interface removed and well washed (Harris & Ukaejiofo 1970).

Erythrocyte contamination of lymphoid cell suspensions was remedied by the addition of isotonic ammonium chloride solution which selectively lyses the red blood cells leaving the lymphoid cells intact both physically and functionally (Boyle 1968, Zatz & Lance 1970). Before use the cells were well washed in medium to remove red blood cell remnants.

4.7 White blood cell counting

1. Lymphocyte suspensions

Cells (usually diluted 1:9 with medium) were counted in a Mod-Fuchs Rosenthal haemocytometer using phase contrast optics.

2. Whole blood

A blood sample was drawn up into a white blood cell pipette and diluted with white blood cell diluent. The pipette was shaken well for 30-45 seconds to lyse all the red blood cells and the resultant white blood cells were counted in a haemocytometer as above.

4.8 Cytocentrifugation

0.5ml samples containing about 5×10^5 cells were loaded into a Shandon cytocentrifuge and spun at 600rpm for 5 minutes. The resultant smears were immediately fixed in absolute methanol for 5 minutes and stained as described.

4.9 Fluorescent antibody staining

Fluorochrome labelled antibodies were used in the identification of B & T cells. For the former, labelled anti-mouse IgG was purchased from Nordic Immunologicals. The fluorochromes were either fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC). For T cell labelling, anti-Thy 1.2 serum was reacted with TRITC Isomer R (Nordic). Free TRITC was removed by passage of the conjugate through a small column of Sephadex G-10.

The labelled antibody was added to cells at an appropriate predetermined titre and allowed to incubate for 30 minutes at 2°C. This was followed by 4 to 6 washes in excess medium to remove unbound antibody. Alternatively, the cells were made up to 1ml with medium and were layered on to 1ml of 15% Ficoll in saline. Following centrifugation for 5 minutes at 800g., the Ficoll layer was diluted with excess medium and the cells spun down to a pellet. The latter method had the advantages of speed and minimal cell loss.

Cells were viewed under 50x oil immersion with a Vickers M41 microscope fitted with epi-illumination from a HBO 200W mercury arc lamp and appropriate filters for each fluorochrome. Only cells showing ring or cap surface staining were counted as positive. Results were expressed as percentage labelling of the total.

4.10 Measurement of intercellular adhesion

Intercellular adhesion was measured by the Couette viscometer method introduced by Curtis (1969). Briefly, about 1.5×10^6 well-washed cells per ml were put into the chamber of the viscometer and allowed to aggregate under a constant shear rate of 10 sec^{-1}. Samples were taken at 0, 7, 14, 21 and 28 minutes and the total particle number in each was assessed in a haemocytometer. The collision efficiency of the cells in the sample was calculated from the formula given by Curtis. The viscometer was siliconised at frequent intervals.

4.11 Protein determination

Protein levels in solution were determined using a method modified

from Lowry et al (1951) and the optical densities measured at 660nm with respect to bovine serum albumin fraction V standards. The unknown protein levels were calculated by linear regression analysis from the standard levels.

4.12 Manufacture and assay of interaction modulation factors (IMFs)

Thymocyte IMFs were produced in the following manner. Cells were suspended at 5×10^7/ml in serum-free medium (HH199 or RPMI) and allowed to incubate for 1.5-2 hours at 37°C. During this time the viability of the cells did not fall significantly. The supernatant obtained after centrifugation at 600-700g. was then filtered through a sterile 0.22µm Millipore filter and subsequently through an Amicon UM10 pressure dialysis membrane at 55p.s.i. of nitrogen. The resulting filtrate should not contain any components with a molecular weight over 10,000 daltons. This fraction was then concentrated on a UM2 membrane (1,000 daltons cut-off) to nearly a tenth of its original volume. At this stage, the semi-pure factor was assayed for its ability to reduce the adhesion of the opposite cell type as described below. Following a successful assay, the factor was further purified on a Biogel P-30 column equilibrated with 10mM HEPES water at pH 7.4. The resulting protein peak was taken and reassayed for de-adhesion activity and usually contained 150-200µg of protein per ml.

The details of the assay system and calculation are given in Curtis & de Sousa (1975). The activity of the factors is expressed in units such that 10^4 units/ml depresses the adhesion of 10^6 B cells to zero.

This activity unit refers to the adhesiveness measured in HH199 medium. Before the factors were used in vivo, they were brought up to physiological salt strengths with the chloride salts of sodium, potassium, calcium and magnesium in the proportions given for HEPES buffered salt solution in the media section (4.1). Factors were stored at -20°C and frozen-thawed no more than once before use.

4.13 Radioactive labelling and cell injection

All radioactive substances were obtained from the Radiochemical Centre, Amersham.

1. Chromium-51. Half life 28 days, supplied as sodium ^{51}chromate in isotonic saline (CJS 1P). Cells were labelled at 50μCi/10^8 cells/ml of medium for 30 minutes at 37°C, followed by at least three washes in excess medium to remove un-utilised radioactivity. At this level, ^{51}chromate labels all cells by covalently binding to proteins but whether the labelling is equal amongst all cell types is not known.

2. Tritiated uridine. Half life 12.25 years, supplied as [5-^{3}H] uridine in aqueous solution (TRA 178). Cells were labelled with 5-25μCi/5 x 10^7 cells/ml of medium for 60 minutes at 37°C with intermittent shaking. Following labelling, the cells were thoroughly washed as above.

3. Tritiated thymidine. Supplied as [6-^{3}H] thymidine in aqueous solution (TRA 61). Cells were labelled with 1μCi/5 x 10^7 cells/ml of medium for 60 minutes at 37°C with

shaking followed by washing in normal medium. The cells were subsequently chased in medium containing 1.5mg/ml of non-radioactive thymidine (Sigma) for 60 minutes at 37°C and washed again.

For injection into animals, radio-labelled cells were suspended at 1.2×10^7 cells/ml of medium and 0.25ml was injected intravenously. At certain times after injection, animals were killed by exsanguination and the following organs were removed for assay of radioactivity: spleen, lungs, liver, peripheral lymph nodes (inguinal, axillary, brachial), mesenteric lymph nodes, bone marrow (as both femurs), small intestine (complete), kidneys, thymus, tail and a sample of blood. The results were expressed as percentage recovery of radioactivity compared to the input. A recovery of greater than 1% in the tail indicated that the injection was faulty and mice exhibiting such recoveries were discarded from the results. The body weight for each mouse was measured and used to calculate a figure for the total blood volume (hence total radioactivity found in the blood) using a formula derived from Kaliss & Pressman (1950); total blood recovery = weight of mouse x 0.121 x recovery/ml in blood sample.

4.14 Radioactive counting techniques

1. Gamma Counting

Samples were loaded into plastic tubes and counted dry in an ICN Tracerlab Gamma Set 500 or Wilj 2001 sodium iodide crystal counter. The counting efficiencies of these machines were about 3% and 1.8% respectively which compares very favourably with theoretical and practical values (Sheppard &

Marlow 1971, Herscowitz & McKillip 1974). Using the ICN counter the background count was usually around 15cpm and samples were counted for one or three minutes. The Wilj counter had a background of 130cpm and samples were counted for five minutes. Counting samples for one minute was valid in all cases where the count exceeded 300cpm as in this instance, the standard deviation is only 3% of the mean.

2. Beta counting

Tissue samples were prepared as suggested by Ford & Hunt (1973) using Aquasol (New England Nuclear) as a scintillant. It was necessary to leave all samples for a few days prior to counting to allow chemiluminescence from the preparation technique to die down. In fact, this phenomenon could be remedied by the addition of 0.4ml sheep red blood cells which neutralises the effect of the hydrogen peroxide used (Ford 1978).

Cell samples were prepared by resuspending a cell pellet in 1ml of 10% ammonium hydroxide and leaving it overnight to digest. The following day 10ml of Aquasol scintillation fluid was added and the samples counted.

Beta emission was measured in Beckman LS200B, Packard Tricarb or Intertechnique scintillation counters with tritium efficiencies around 60%. In the former counter type, counting proceeded until a fixed error was achieved; in the latter two, counting was for one minute only.

4.15 Measurement of the toxicity of thymocyte IMF 127

LNL were labelled with 100μCi ^{51}Cr/5 x 10^7 cells/ml of medium for 60 minutes at 37°C, followed by two washes in RPMI/10%FCS. After a 30 minute incubation at 2°C, the cells were washed a further three times in RPMI/5%FCS. Cells were then resuspended according to the following regime:

Control - 1.2 x 10^7 cells/ml in RPMI/10%FCS.

Exptal. - 1.2 x 10^7 cells/ml in RPMI/10%FCS containing 945,000 units (63μg) T IMF 127 per ml.

After a 15 minute incubation at room temperature, both samples were washed once in RPMI/5%FCS and resuspended to 1.2 x 10^7 cells/ml. Samples were then incubated at 37°C, with occasional shaking and 0.1ml samples were taken at appropriate time intervals.

The 0.1ml samples were diluted to 0.5ml with medium, centrifuged at 400g. for 10 minutes and 0.1ml samples of the supernatant removed for assay of released radioactivity.

The maximum release of radioactive chromate was assessed by exposing an aliquot of cells to 5% Lubrol PX (Sigma) for 30 minutes followed by processing in the normal manner.

The results are expressed according to the formula -

$$\frac{\text{Recovery in 0.1ml supernatant (Cont. or Exp.) - Background}}{\text{Recovery in 0.1ml supernatant (Lubrol treated) - Background}} \%$$

4.16 Sectioning of organs

Organs were removed and fixed in formol-saline. Following dehydration

and clearing through chloroform to xylene, the organs were embedded in Paraplast Tissue Embedding medium and 4-5μm sections cut on a Jung rotary microtome. The sections were floated out on hot water and dried out on to albumen-treated subbed slides.

4.17 Autoradiography

Sections were dewaxed and rehydrated to water. Dipping in emulsion followed a method modified from Rogers (1969). Slides were dipped in a 1:2 or 1:3 dilution of Ilford Nuclear Research Emulsion (K2 or K4) in distilled water at 50°C. After being allowed to drain for several seconds, the slides were left to dry horizontally for one hour on a cooled tray in total darkness. The coated slides were then put into plastic racks in sealed containers with a small amount of silica gel and left to expose at 2°C for 3-6 weeks. Following development in Ilford D19 and fixing in Amfix (May and Baker), the slides were stained as described in 4.18.

4.18 Staining

Sections - these were dewaxed and rehydrated prior to staining by either of the methods below:

1. Methyl green-pyronin Y-water (15:25:60), differentiated in n-butanol.
2. Harris' haematoxylin and eosin Y, differentiated in acid alcohol or calcium chloride solution and "blued" in 1% sodium bicarbonate.

Sections were then rehydrated, cleared with clove oil and mounted in Canada balsam.

Smears - these were fixed in absolute methanol and allowed to dry. Two different staining methods were used:

1. Leishman's. Smears were stained in 100% Leishman's, transferred to 50%, differentiated in buffered water at pH 6.8 and allowed to dry.
2. May-Grünwald-Giemsa. Smears were stained in 100% followed by 50% May-Grünwald, followed by 10% Giemsa and were differentiated in distilled water.

4.19 Counting of autoradiographs

Sections prepared for autoradiography, as previously described, were examined under 250x magnification using Leitz Heine dark-field illumination. Only cells with more than 20 grains were counted as positive and their position in the organ examined was then determined using bright field optics. At least 300 cells were counted for each experiment.

4.20 Statistical analyses

Results are routinely expressed as arithmetic mean plus or minus the sample standard deviation. Comparison between control and experimental data was done using Student's t test, the variance ratio (F) test having been previously done to check the compatibility of the data. However, this test cannot be used if the variances of the two samples being compared are significantly different. In this case, Cochran's modified t' test (1964) must be used.

t' is given by the formula:

$$t' = \frac{\text{Mean 1 - Mean 2}}{\sqrt{k_1 - k_2}}$$

where $k_1 = \frac{(\text{st. dev.}_1)^2}{n_1}$

and $k_2 = \frac{(\text{st. dev.}_2)^2}{n_2}$

This gives a value for t' for which the significance level must now be calculated. To do this, the significance levels desired (e.g. 1% or 5%) are looked up in the t tables for $(n_1 - 1)$ and $(n_2 - 1)$ degrees of freedom. These values are called t_1 and t_2 respectively. The t'' value for this particular level of significance is given by:

$$t'' = \frac{(k_1 \times t_1) + (k_2 \times t_2)}{(k_1 + k_2)}$$

if t' is greater numerically than t'' then the data is significant within the significance levels chosen.

CHAPTER FIVE

EXPERIMENTAL PROTOCOLS

CHAPTER FIVE

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1. The effect of chromate on the localisation of labelled thymocytes.

The level of chromate in the normal radioactive labelling solution is approximately 0.1mM. [^{3}H] uridine labelled thymocytes were incubated for 30 minutes at 37°C and 10^{8} cells/ml in saline containing 150µl of 0.1mM potassium chromate. Control cells were incubated in the same way in saline without chromate. Cells were then washed and injected as normal and after 3.5 hours organs were assayed for radioactivity by scintillation counting as described in the Methods section.

2. Localisation patterns of ^{51}Cr labelled red blood cells.

Labelled red blood cells at 3×10^{6} cells/0.25ml were injected intravenously and organs were assayed for radioactivity after 15 minutes and 4 hours.

3. Localisation patterns of normal, glutaraldehyde fixed and heat-killed labelled thymocytes.

Thymocytes labelled with ^{51}Cr were resuspended to 1.5×10^{7} cells/ml.

(a) Control cells. Cells were incubated for 30 minutes at 37°C in normal RPMI.

(b) Glutaraldehyde fixed cells. Cells were incubated in glutaraldehyde solution for 30 minutes at 37°C.

(c) Heat-killed cells. Cells were incubated for 30 minutes at 56°C in RPMI containing 20µg/ml DNase.

All cell types were then washed 3 times in fresh medium and their viabilities checked by dye exclusion prior to injection. Each mouse was given 3×10^6 cells in 0.25ml of medium and organs were assayed for radioactivity after 2 hours.

4. Injection of free sodium ^{51}Cr chromate.

This stock was obtained from the supernatant of the first wash of cells following normal labelling procedures. The radioactivity will be in the form of free ^{51}Cr-chromate ions or ^{51}Cr-chromate bound to small peptide units released from cells during the 30 minute incubation. 0.5ml volumes of this supernatant were injected subcutaneously or intraperitoneally and organs were assayed for radioactivity after 30 minutes and 4 hours.

5. Alternative injection routes of cells.

^{51}Cr labelled thymocytes, at 6.7×10^7 cells/0.5ml medium, were injected subcutaneously or intraperitoneally and organs were assayed for radioactivity after 30 minutes and 4 hours.

6. The adhesive and localisation properties of normal thymocytes and nylon wool filtered thymocytes.

Filtered thymocytes were obtained by the nylon wool method described. The adhesiveness of normal and filtered thymocytes was measured by the Couette viscometer method.
Both cell types were labelled with ^{51}Cr and injected into syngeneic recipients. Their respective organ localisations were measured at 15 minutes, 30 minutes, 1, 2, 4 and 24 hours after intravenous injection.

7. The filtration of thymidine labelled thymocytes through nylon wool columns.

Thymocytes were labelled with [^{3}H]thymidine and passaged through nylon wool columns in the manner described. Cells were eluted in 2ml fractions and the cell number and radioactivity in each fraction were determined.

8. Comparison of localisation patterns of cyclophosphamide (CPP) produced T lymphocytes and nylon wool filtration produced T lymphocytes.

^{51}Cr labelled CPP T cells and column T cells were produced as described in the Methods section. Both cell types were injected into syngeneic recipients which were killed at 15 minutes, 30 minutes, 1, 2, 4 and 24 hours after injection. Organs were then assayed for radioactivity.

9. The effect of the number of cells injected on the recovery of ^{51}Cr labelled thymocytes and lymph node lymphocytes in various organs.

^{51}Cr labelled cells were produced as described and resuspended at the appropriate concentrations in 0.25ml of medium, which was injected intravenously. Various organs were assayed for radioactivity after 1 hours.

10. The effects of large quantities of unlabelled cells on the localisation patterns of ^{51}Cr labelled lymphocytes.

The number of unlabelled cells added varied for each experiment.

However, the standard inoculum contained 3×10^6 labelled cells in 0.25ml and unlabelled cells were added to this without changing the final volume. Mixtures were allowed to incubate for 15 minutes at 37°C prior to injection. Both ^{51}Cr and [^{3}H] uridine were used as labels and organs were assayed for radioactivity after set times. Some organs were also processed for autoradiography.

11. Intravenous injection of thymocyte IMF.

Before use the protein level and activities of IMFs were measured by the techniques described. For injection, thymocyte IMF was made up to 80,000 units/ml of buffered saline and 0.25ml was injected intravenously. Control animals were given 0.25ml saline only. After certain time intervals, primarily 2 and 24 hours, blood was withdrawn into heparinised syringes and the spleens were removed. The blood samples were treated in the following ways:

(a) After dilution in a white blood cell pipette the total leucocyte count per ml of blood was measured.

(b) Preparation of a leucocyte rich smear, as described, to obtain a differential count of leucocyte types.

(c) Separation of blood lymphocytes and staining with fluorescent antibody to obtain the percentage of Ig positive cells.

The spleens were treated in two ways:

(a) For histology by routine methods.

(b) Separation of spleen lymphocytes and measurement of Ig positive lymphocyte level as with the blood.

As many cells as possible were counted for each result and the data is that pooled from at least ten animals.

12. The effect of thymocyte IMFs on labelled thymocyte localisation.

^{51}Cr or $[^{3}H]$ uridine labelled cells were incubated with appropriate amounts of thymocyte IMF for 15 minutes at room temperature prior to injection (3×10^{6} cells in 0.25ml injected with factor). The following IMFs were used:

TF122 at 7,000 units (13µg protein) per injection.

TF124 at 25,000 units (14µg protein) per injection.

TF126 at 21,000 units 16µg protein) per injection.

In one series of experiments using TF126, the cells were washed free of IMF prior to injection.

After set time intervals, organs were assayed for radioactivity.

CHAPTER SIX

RESULTS

CHAPTER SIX

RESULTS

Radioactive chromate has, in the past, been criticised as a cell label for transfusion studies. These first few experiments were designed to examine the effect of chromate on the cell surface with respect to localisation patterns, where excess label is found if released, and where dead labelled cells localise. Answers to these questions are necessary in the interpretation of the remaining experiments.

6.1 The effect of chromate on the localisation of labelled thymocytes (see Table 1)

This experiment compares the localisation patterns of thymocytes labelled with tritiated uridine before and after treatment with non-radioactive potassium chromate at a similar concentration as would be used for radioactive labelling.

Due to the preparation technique for scintillation counting, the variances of the data are rather high but despite the tendency for treated cells to accumulate in the liver to a slightly greater extent, there is no evidence to suggest that this is significant.

6.2 The localisation patterns of ^{51}Cr labelled red blood cells. (see Table 2)

By labelling red blood cells, the relative blood contents of the sampled organs at 15 minutes and 4 hours after injection can be estimated. The total recoveries of all the sampled organs are very high, 100% and 86% at 15 minutes and 4 hours respectively, which would tend to indicate some inaccuracy as many organs containing appreciable blood

were not sampled. It is interesting to note the recovery in the lymph nodes which is at least detectable compared with the data from table 3 on the localisation of dead cells. The lung levels give an idea of the number of cells likely to be embolised or trapped following intravenous injection.

6.3 The localisation patterns of glutaraldehyde fixed and heat-killed thymocytes (see Table 3)

1. Glutaraldehyde fixed cells. These cells still apparently maintained a high viability as measured by dye exclusion, although most cells were probably no longer alive by other criteria. Most cells end up in the liver if they do not become trapped in the lung capillary beds after injection. The lymph node recovery is undetectable (below 0.01%) and the recoveries in the spleen and kidneys are probably due to the trapping of previously untrapped dead cells.

2. Heat-killed cells. The patterns of recovery are the same as with the glutaraldehyde fixed cells. The total recovery is surprisingly high, being greater than with normal cells.

6.4 Utilisation of free injected chromate. (see Table 4)

The injected chromate would be in the form of free ^{51}Cr chromate or ^{51}Cr chromate bound to small peptide units. The pattern of recovery depends on the route of injection, with most of the radioactivity ending up in the liver, small intestine, kidneys and blood.

6.5 Alternative injection routes of labelled cells (see Table 5)

1. Intraperitoneal injection. The recovery of cells injected by this route is minimal even after 4 hours. The high levels recorded for the blood are probably an artefact as a result of the collection technique.

2. Subcutaneous injection. Very few, if any, cells ever get into circulation from the subcutaneous route. The levels seen may be due to released label as the cells are often damaged from this type of injection.

6.6 The localisation and adhesive properties of normal thymocytes and thymocytes filtered through nylon wool.

1. Adhesiveness (see Table 6)

The average mutual adhesiveness of unfiltered thymocytes is much less than that of filtered cells. The large variance in the data for the filtered subpopulation is probably due to the variability of the population of cells eluted from the column. Only 50% of the cells put on to the column were recovered in the eluate.

2. Localisation patterns (see Tables 7, 8, Figure 1)

The time course data is expressed on Table 7 and in Figure 1 and the results of the t tests on Table 8.

Spleen - the localisation of both cell types increases rapidly over the first four hours, although the filtered subpopulation displays a significantly lower localisation throughout. At 24 hours, the percentage

recovery has decreased slightly and the difference between the entire thymus population and the filtered cells is probably not significant except that the variance of the control data is very high. The rate of entry of the filtered cells into the spleen over the first 30 minutes may well be depressed.

Lungs - both cell types localise in the lungs at early time intervals but the levels rapidly drop off. The recovery of the filtered cells is significantly lower over the first 2 hours but by 4 hours, both cell types exhibit an equally low recovery.

Liver - the liver recovery remains largely constant with time for both cell types but up to 4 hours the recovery of filtered cells is again significantly depressed. After 24 hours, this difference is no longer apparent.

Lymph nodes - recovery in the lymph nodes of both control and filtered cells is low and probably for this reason there are no significant differences in the recovery at most times. The initial rate of entry of filtered cells into the lymph nodes may well be depressed.

Bone marrow - again the percentage recovery in the bone marrow after injection of either cell type is low but the recovery of filtered cells is significantly lower over the first four hours.

Blood - the recovery of both cell types in the blood starts off at a high level and decreases rapidly with time. However, at times up to and including 2 hours, the blood recovery of filtered cells is significantly higher than that of control cells.

Small intestine - the recovery of both cell types is low and at most time intervals there is no significant difference between the recoveries.

Kidneys - the recoveries in the kidneys of both cell types remain high throughout with the filtered cells being recovered in significantly higher levels at all time intervals, including 24 hours.

Total recovery - despite a tendency at early time intervals for the total recovery of control cells to be greater than that of filtered cells, the difference in the majority of cases is non-significant.

No detectable recovery was found in the thymus with either cell type at any time interval (data not included).

6.7 The filtration of thymidine labelled cells through nylon wool columns

Thymocytes used had been labelled with tritiated thymidine as described.

The results (Table 9) show that the cell yield from the column is about 58% and the yield of thymidine labelled cells is very similar. Thus, thymidine labelled cells are not significantly passaged or retained. However, should this be the case, the cpm per 10^7 cells should remain a constant. There is a tendency for the fifth fraction to have a slightly higher cpm per 10^7 cell recovery but this is probably due to higher variances in counting such a small number of cells.

6.8 The localisation of cyclophosphamide produced and nylon wool produced T cells.

Since it had been found that passage of thymocytes through a nylon wool column caused the selection of a specific subpopulation, it was interesting

to see whether the same could be found for peripheral T cells produced by similar means. CPP was used to produce a "control" population of T cells to be compared with T cells produced by nylon wool filtration of lymph node lymphocytes.

Summary of localisation patterns of injected cells. (Tables 10, 11, 12 Figure 2)

1. Spleen. Over the first hour after injection, both cell types localise rapidly in the spleen, although the column T cells are slightly slower since the level at 15 minutes is significantly lower. After one hour, the CPP T cells reach a maximal level and gradually fall off to a slightly lower level at 24 hours. The column T cells do not reach a maximal level until 2 hours and this level, significantly greater than the CPP cell level, is maintained until 24 hours when both values are identical.

2. Lungs. The initial level in the lungs of column T cells is much higher than that of CPP T cells but falls off more rapidly, such that a constant low level is reached in both cases at around 4 hours. Although the initial difference is of the order of 13%, it is not significant due to the large differences in variances of the data.

3. Liver. Initially, the level of both cell types is high in the liver, but the level of column derived cells falls to around 10%. The CPP T cells are retained in the liver longer over the first hour, but subsequently fall to similar values as the column T cells at later times. At 24 hours, both liver levels have increased further but at all times the recovery of column T cells is lower.

4. Lymph nodes. From pooled mesenteric and peripheral lymph nodes. The increase in lymph node localisation with time is broadly similar with both cell types, although there is a slight lag of lymph node entry by column T cells around 2 hours. After 24 hours, both levels are identical.

5. Bone marrow. Although both cell types localise to the same extent in the bone marrow at an early time, the rate of entry of CPP T cells is slower over the next 2 hours. After 4 hours, levels of both cell types are the same.

6. Blood. At most time intervals up to and including 24 hours after injection, the level of CPP T cells in the blood is significantly greater than that of column derived T cells. However, by 2 hours this level is only 4% maximum, dropping off slowly with time. The final level of column T cells in the blood is very low.

7. Small intestine. The levels and rates of entry of both cell types into the small intestine is very similar.

8. Kidneys. As with the small intestine, both levels and rate of entry are similar.

6.9 The effect of cell number on the organ localisation of injected LNL and thymocytes (see Table 13)

This experiment was performed to see whether the injection of larger cell numbers caused non-specific trapping in various organs. The low cell dose was 3×10^6 cells per animal (the dose normally used in all the experiments reported here); the high cell dose was $3\text{-}3.5 \times 10^7$ cells per animal - both in the same volume of medium.

1. Recovery of lymph node lymphocytes in high and low doses.

Although, in the data presented here, there are no significant differences, administration of the higher dose resulted in marginally lower recoveries in the peripheral lymph nodes.

2. Recovery of thymocytes injected in high and low doses.

As with the above, apart from marginal, non-significant differences, the recoveries of high and low doses are the same.

Note - the results for the low level injection of lymph node lymphocytes do not correspond to other data from similar cells derived from the same source. For this reason, the comparison is made only between animals injected with the same cell inoculum on the same day, since, for some unknown reason, spleen-seeking cells may be enriched in certain samples.

6.10 The effect of large quantities of unlabelled cells on the localisation of labelled lymphocytes.

The maximum inoculum cell number was 3.3×10^7 cells, a dose which has already been shown not to "clog" up the recirculatory system.

1. Labelled LNL with ten times the number of unlabelled LNL (Table 14)

This control experiment shows that the addition of a large number of unlabelled cells, identical to those of the labelled population, makes no difference to the organ distribution of labelled cells at 4 hours.

2. Labelled LNL with ten times the number of unlabelled thymocytes (Table 14)

There is a significantly increased localisation of labelled cells in the spleen compared to the control at 4 hours. This is accompanied by a tendency for increased localisation in the liver, although the difference is not significant. The most interesting observation is that the total recovery of radioactivity from all the organs sampled is greater in the thymocyte-added injection compared to the control. This argues against the proposal that the increased localisation in the spleen is at the expense of localisation to other organs. The increased total recovery indicates that the extra cells have been sequestered from some other location other than those sampled. At 24 hours, the differences previously seen are no longer apparent.

Experiments using tritiated uridine labelled LNL with unlabelled thymocytes indicate that the increased localisation in the spleen is present as early as 15 minutes after injection and is maintained for at least 24 hours (Table 15). The latter result is at variance with the data using ^{51}Cr labelled cells.

Autoradiographical analysis (Table 16) of the distribution of $[^{3}H]$ uridine labelled LNL in the spleen with unlabelled thymocyte treatment indicates that this population enters the white pulp more rapidly than the control population, which remains in the red pulp for a longer time. At 15 minutes and one hour after injection, an increased number of cells are found in the marginal zone of the white pulp. At 24 hours, any differences previously seen are no longer apparent.

3. Labelled LNL with twice the number of unlabelled lymph node B lymphocytes (Table 17)

At 30 minutes after injection, there is no difference between the localisation of normal labelled LNL and LNL injected with twice that amount of unlabelled B lymphocytes also derived from lymph nodes. However, at 4 hours after injection, significant decreases are seen in the spleen and blood localisation and increases in the bone marrow and lymph node localisation compared to the control situation. Other experiments (Table 18) have indicated a qualitatively similar rise in lymph node localisation with little effect on the bone marrow, blood and spleen localisation but an increase in the liver localisation. This feature may be apparent in the data for 30 minutes after injection, but due to the large variances in the data, comparisons cannot be made.

4. Splenic lymphocytes with three times the number of unlabelled thymocytes (Table 19)

At 4 hours, the only significant difference is a decrease in localisation of treated cells in the liver with a tendency towards an increase in the lymph nodes. The drop in the liver is also reflected in a drop (though not significant) in the total recovery.

5. Splenic B lymphocytes with five times the number of unlabelled thymocytes (Table 20)

The localisation of labelled cells in the lymph nodes at 4 hours is significantly decreased, although, in any case, the control values are low. The localisation of treated labelled cells in the blood and spleen is also

decreased and the level in the liver increased. Although none of these differences are significant, they are sufficient to account for the significant decrease in the lymph nodes.

6.11 Properties of thymocyte IMFs manufactured and used (see Table 21)

From the data provided in the table, it seems that there does not appear to be any close correlation between the protein concentration and the activity of the IMF in depressing B lymphocyte adhesion. This may be due to the differences in purity of the individual preparations or that the protein part of the molecule is not the active region.

6.12 Cytotoxicity of thymocyte IMF 127 (see Table 22)

This particular IMF has a very high activity for protein content and thus although the cells in the experiment are treated with a similar weight of protein as in other experiments, they are in this case subjected to a much higher B lymphocyte deadhesion activity. Over the 4 hour time course of the experiment, there is no indication that the IMF is particularly toxic and even after 4 hours, the viability by dye exclusion is still greater than 90%.

6.13 The effect of intravenous injection of thymocyte IMF on the lymphoid system. (See Table 23)

1. White blood cell counts. The number of white blood cells per ml of blood increases significantly after 2 hours following injection of IMF. The differential white blood cell count from smears suggests that the increase is due to a non-specific leucocytosis involving lymphoid,

granuloid and other mononuclear cells. Whether there is also an increase in the red blood cell count is not known. The leucocytosis is detectable as early as 15 minutes after injection of the IMF.

After 24 hours, the leucocytosis is not apparent and the differential white blood cell count indicates that all white blood cell types are lost in proportion, such that control and experimental differential counts are the same. The large difference in the differential count data at 2 and 24 hours is very hard to explain. It is possible that the preparation techniques varied on the separate occasions but this is unlikely to explain such a large difference.

2. Percentage of B cells in blood and spleen. Measurement of the percentage of B cells (as surface immunoglobulin positive cells) in the blood and spleen shows an increase in the B:T cell ratio in the former and decrease in the B:T ratio in the latter at 2 hours. Thus, the spleen B:T ratio goes down and the blood B:T ratio goes up. The difference in the spleen can be explained either by a loss of B cells or gain of T cells each of which will affect the B:T ratio in the same way.

3. Histology of the spleen after IMF treatment. At 2 hours after IMF injection, the red pulp of the spleen appeared more acellular than in the control. Whether the white pulp was also depleted was impossible to quantify, although there was no marked difference visible. After 24 hours, the red pulp appeared to have regained its normal cellularity.

The lymph nodes were also examined in this study. There was no

difference in the B:T cell ratio at any time and no apparent histological alterations.

6.14 The effect of thymocyte IMF on lymphocyte localisation

As has already been mentioned, it has been found that mice from different sources display different organ recoveries after injection of a similar cell type; this fact is very evident from the 4 hour control data shown in tables 14 and 17.

1. General notes on the trend of lymph node lymphocyte localisation with time.

At one hour after injection, the number of cells localising in the spleen has almost reached a maximum, a level which is maintained and gradually decreases to the lower 24 hour value. A similar pattern is seen in the liver localisation. The lymph node levels increase with time almost linearly - it is not known whether the increase after 24 hours continues in the same way, as no experiments were performed for longer than 24 hours. The blood level falls with time as does the total recovery from all the organs. Localisation in the small intestine increases with time. The kidney and bone marrow levels reach an early maximum and then remain largely unchanged with time.

2. The effect of thymocyte IMF122 on lymph node lymphocyte localisation.

TF122 (7,000 units) 1 hour. Although there are probable increases in the spleen and liver localisation and total recovery, these are not statistically different due to the lack of data.
Table 24.

There is, however, a significant drop in the recovery in the peripheral lymph nodes which is not apparent in the mesenteric lymph nodes.

4 hours. The decrease in the localisation in the peripheral lymph nodes seen at one hour is still present at 4 hours along with significant increases in localisation in the spleen and liver. There is also a decrease in localisation in the bone marrow. It should be noted that, again, localisation in the mesenteric lymph nodes is unaffected and the level of labelled cells in the blood does not show a tendency to be decreased. The total recovery of treated cells in all the organs sampled is again elevated.

24 hours. Any differences seen in the previous time intervals are no longer apparent at 24 hours. The total recovery of treated cells is also identical to that of the control.

3. The effect of thymocyte IMF124 on lymph node lymphocyte localisation.

TF124 25,000 units. Table 25.

The results for the experiment at 4 hours are qualitatively similar to those at 4 hours in table 24 and the mice used in both experiments were derived from the same source. Treatment with T IMF124 in this case does not cause a significant increase in the localisation in the liver. The remaining data in this table shows the effect of a thymocyte conditioned medium (10^8 cells/ml for 60 minutes at 37°C) on LNL localisation.

Although the data is not significantly different, the trend is towards a similar effect to the purified IMF.

Using the same IMF, the experiments were repeated at 2 and 8 hours (Table 26). This data is totally incompatible with that previously described due to the different source of animals for the experiments. For this reason, the effect of T IMF124 over a time course cannot be adequately documented. At 2 hours, there is an increase in the liver and decrease in the peripheral lymph nodes, as in the other data. However, a previously un-noted decrease in the mesenteric lymph nodes is also seen. At 8 hours, the difference in the liver is still maintained but the lymph node data, although remaining apparently lower, is not significantly different. It should be noted at both times that the total recovery in IMF treated animals is higher than in the controls.

4. The effect of thymocyte IMF124 on the intrasplenic distribution of $[^3H]$ uridine labelled LNL (See Table 27)

This experiment was carried out under the same conditions as that documented on table 16 on the effects of unlabelled thymocytes on the intrasplenic distribution of LNL. In the latter experiment, at 15 minutes after injection, labelled cells which had been treated with thymocytes were seen to enter the white pulp more rapidly. The same is found for IMF treated cells, though to a greater extent. Cells enter the white pulp more rapidly but appear to be transiently held up in the marginal zone of the white pulp. At one hour the hold-up in the marginal zone is still apparent, while at 24 hours no difference in the

distribution of control or IMF treated cells is seen. It should also be remembered that the differences shown in the table are from results expressed as a percentage of total counted. In fact, since the recovery in the treated animals is always greater, the differences are greater than are indicated on the table.

5. The effect of thymocyte IMF126 on lymph node lymphocyte localisation

TF126 21,000 units. Table 28

The data shows the effect of both intravenous injection of the IMF on the localisation of labelled LNL and the effect of IMF treatment of the cells, followed by one wash in medium prior to injection, on the localisation of labelled LNL.

Intravenous injection of T IMF with LNL. In this instance, the IMF causes a significant decrease in the localisation to both peripheral and mesenteric lymph nodes with a concomitant increase in the liver. Contrary to other results, there is no increase in the spleen and a significant decrease in localisation in the small intestine. The total recovery is similar to the control.

Treatment of LNL with T IMF prior to injection. This parallels the results of the intravenous injection of IMF with a tendency for increased localisation in the spleen and a smaller increase in localisation to the liver. In both the treatments, the levels of cells in the blood are similar.

6. The effect of thymocyte IMF122 on spleen B lymphocyte localisation.

Table 29 7,000 units TF122

2 hours The only significant increase is in the liver while the decrease in the peripheral lymph nodes is not significant due to lack of data.

4 hours The increase in the liver is still apparent but is not significant due to the large variance of the data. Although the lymph node recoveries are very low, there is a definite decrease in the localisation in both peripheral and mesenteric lymph nodes similar to that seen with the excess thymocyte treated spleen B lymphocytes (Table 20). In both the 2 and 4 hour time courses the increase in the liver is also reflected by an increase in the total recovery, suggesting that increased localisation in the liver is not at the expense of localisation elsewhere.

6.15 The localisation patterns of ALS produced LN B lymphocytes

The data shown in table 31 shows the localisation patterns of ^{51}Cr labelled B lymphocytes produced by in vivo ALS treatment. The majority of cells localise in the spleen and liver, although the variability of the data is high despite the large sample number. This data can be compared to that on table 20 of the localisation patterns of ALS produced spleen B lymphocytes. It would appear that the latter cell

type localises more in the lymph nodes than B lymphocytes from lymph nodes. This finding does not support the concept that lymph node cells home specifically back to lymph nodes.

TABLE 1

THE EFFECT OF CHROMATE ON THE LOCALISATION PATTERNS OF ^{3}H URIDINE LABELLED THYMOCYTES

Mouse Source - ARC

At 3.5 hours

	SPLEEN	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY	n
Control	16.82 ± 5.03	26.6 ± 6.08	1.23 ± 0.85	1.27 ± 0.78	7.4 ± 0.6	7.5 ± 2.04	60.85 ± 2.77	4
Chromate treated *	15.62 ± 3.36	29.8 ± 7.66	1.63 ± 1.42	1.83 ± 1.52	8.7 ± 1.2	8.7 ± 3.2	66.37 ± 7.47	4

* See Experimental Protocol

No significant differences between control and treated cells.

TABLE 2

THE LOCALISATION PATTERNS OF INTRAVENOUSLY INJECTED ^{51}Cr LABELLED RED BLOOD CELLS

Mouse source - ARC

TIME HOURS	SPLEEN	LUNGS	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
0.25	2.58	29.72	24.67	0.1	0.13	0.5	34.24	1.24	6.38	100
4	5.69	12.51	31.49	0.07	0.05	1.16	24.78	0.94	8.37	86

TABLE 3

THE LOCALISATION PATTERNS OF NORMAL, HEAT-KILLED AND GLUTARALDEHYDE FIXED ^{51}Cr LABELLED THYMOCYTES INJECTED INTRAVENOUSLY

Mouse source - ARC

At 2 hours.

	SPLEEN	LUNGS	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
Normal	13.45 ± 2.84	15.05 ± 1.66	29.68 ± 0.03	0.16 ± 0.05	0.25 ± 0.04	0.97 ± 0.03	2.6 ± 0.37	0.64 ± 0.03	3.11 ± 0.03	67.7 ± 1.94
Heat-Killed *	2.95 ± 0.23	31.02 ± 0.26	39.3 ± 0.63	0	0	0.48 ± 0.11	3.41 ± 0.54	0.33 ± 0.08	3.2 ± 0.15	81.0 ± 1.02
Glutaraldehyde fixed *	1.56 ± 0.01	36.83 ± 0.13	21.75 ± 1.55	0	0	0.37 ± 0.007	3.5 ± 0.13	0.5 ± 0.18	1.56 ± 0.84	66.32 ± 2.27

* See Experimental Protocols

TABLE 4

THE UTILISATION OF FREE INJECTED ^{51}Cr-CHROMATE

Mouse source - ARC

INJECTION ROUTE	TIME (HOURS)	SPLEEN	LUNGS	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
IP	0.5	0.36	0.35	9.4	0.07	0.4	0.4	18.1	5.6	2.5	37.9
IP	4	0.33	0.33	9.08	0.05	0.2	0.5	8.51	4.35	1.32	25.1
SC	0.5	0.1	0.4	2.36	0.28	0.13	0.49	13.7	2.1	2.72	22.9
SC	4	0.1	0.25	2.45	0.15	0.08	0.4	11.9	1.6	1.7	19.2

IP = intraperitoneal

SC = subcutaneous

TABLE 5

ALTERNATIVE INJECTION ROUTES OF ^{51}Cr LABELLED THYMOCYTES

Mouse source - ARC

INJECTION ROUTE	TIME (HOURS)	SPLEEN	LUNGS	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
IP	0.5	0.41	0.27	0.76	0.04	0.03	0.4	19.6	1.5	0.2	23.3
IP	4	1.86	0.21	3.4	0.02	0.06	0.17	6.53	3.6	0.6	16.6
SC	0.5	0.01	0.03	0.1	0.02	0.01	0.07	0.8	0.2	0.2	1.5
SC	4	0.01	0.05	0.15	0.03	0.01	0.1	2.02	0.21	0.24	2.9

IP = intraperitoneal

SC = subcutaneous

TABLE 6

ADHESIVENESS OF CONTROL AND EXPERIMENTAL THYMOCYTES

TYPE	COLLISION EFFICIENCY (%)	± STANDARD DEVIATION (STANDARD ERROR)	n
Control (whole thymus)	3.5	2.62 (0.7)	14
Experimental (subpopulation)	17.1	13.01 (2.2)	35

Significance of difference between control and experimental data, as assessed by Cochran's modified T test, $p < 0.001$

NOTE: medium HH199 + FCS used for adhesion measurements

TABLE 7

THYMOCYTE/THYMOCYTE SUBPOPULATION DATA

TIME (HOURS)	SPLEEN		LUNGS		LIVER		LYMPH NODES		BONE MARROW		BLOOD		SMALL INTESTINE		KIDNEYS		TOTAL % RECOVERY	
0.25	6.14 ± 0.74	3.49 ± 2.4	27.58 ± 5.46	12.81 ± 1.74	24.6 ± 1.73	19.44 ± 1.99	0.43 ± 0.1	0.33 ± 0.11	1.00 ± 0.15	0.77 ± 0.13	10.85 ± 4.2	21.02 ± 6.19	1.37 ± 0.45	1.78 ± 0.25	3.55 ± 0.23	4.72 ± 0.73	73.25 ± 3.47	65.9 ± 5.66
	n=6	n=5	n=6	n=5	n=6	n=5	n=4	n=5	n=4	n=5	n=6	n=5	n=4	n=5	n=4	n=5	n=4	n=5
0.5	10.35 ± 1.26	3.06 ± 1.48	17.86 ± 4.91	10.06 ± 2.69	27.6 ± 4.44	20.75 ± 3.56	0.62 ± 0.08	0.42 ± 0.06	1.25 ± 0.2	0.84 ± 0.13	6.92 ± 1.48	14.96 ± 4.76	1.31 ± 0.25	1.41 ± 0.28	3.30 ± 0.18	4.5 ± 0.21	69.1 ± 3.16	56.5 ± 3.7
	n=5	n=4	n=5	n=4	n=5	n=4	n=4	n=4	n=4	n=4	n=4	n=3	n=4	n=4	n=4	n=4	n=4	n=4
1	12.77 ± 2.3	7.5 ± 4.26	12.78 ± 2.81	5.04 ± 0.94	27.62 ± 2.52	20.22 ± 2.77	0.91 ± 0.19	0.57 ± 0.25	1.9 ± 0.69	0.88 ± 0.15	7.47 ± 3.88	16.22 ± 4.0	1.2 ± 0.1	1.51 ± 0.13	3.01 ± 0.7	4.85 ± 0.73	63.8 ± 1.76	61.3 ± 4.66
	n=5	n=5	n=5	n=5	n=5	n=5	n=3	n=5	n=3	n=5	n=3	n=5	n=3	n=5	n=3	n=5	n=3	n=5
2	14.3 ± 2.78	7.58 ± 1.5	5.32 ± 0.81	3.26 ± 1.02	25.47 ± 3.88	23.67 ± 1.59	0.98 ± 0.21	0.73 ± 0.24	1.77 ± 0.19	1.1 ± 0.28	6.01 ± 3.32	12.18 ± 2.61	1.02 ± 0.13	1.27 ± 0.38	2.63 ± 0.20	4.45 ± 0.46	59.4 ± 4.75	54.2 ± 3.95
	n=6	n=4	n=6	n=4	n=6	n=4	n=4	n=4	n=4	n=4	n=4	n=3	n=4	n=4	n=4	n=4	n=4	n=4
4	17.37 ± 3.4	11.57 ± 1.87	2.07 ± 0.35	1.60 ± 0.63	28.11 ± 1.11	21.13 ± 4.05	1.09 ± 0.23	0.87 ± 0.26	2.01 ± 0.6	1.22 ± 0.16	5.83 ± 2.85	9.21 ± 1.96	0.84 ± 0.22	0.90 ± 0.11	2.58 ± 0.22	4.28 ± 0.47	56.8 ± 1.29	53.9 ± 4.52
	n=6	n=5	n=6	n=5	n=6	n=5	n=4	n=5	n=4	n=5	n=5	n=5	n=4	n=5	n=4	n=5	n=4	n=4
24	10.08 ± 5.7	5.36 ± 0.47	0.31 ± 0.1	0.31 ± 0.1	16.90 ± 3.57	18.15 ± 1.25	0.35 ± 0.09	0.44 ± 0.18	0.67 ± 0.26	0.94 ± 0.32	4.12 ± 0.96	3.26 ± 0.75	0.51 ± 0.11	0.44 ± 0.14	2.83 ± 0.84	4.17 ± 0.25	33.3 ± 9.2	34.1 ± 2.5
	n=8	n=5	n=8	n=5	n=8	n=5	n=4	n=5	n=4	n=5	n=5	n=4	n=5	n=5	n=5	n=5	n=6	n=5

TABLE 8

STATISTICAL EVALUATION (P VALUES) OF DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL ORGAN LOCALISATION

Organ	Time in hours after injection of labelled cells					
	0.25	0.5	1	2	4	24
Spleen	0.03	0.0001	0.04	0.002	0.008	>0.05
Lungs	0.003	0.02	0.0004	0.007	>0.05	>0.05
Liver	0.001	0.04	0.002	>0.05	0.003	>0.05
Lymph Nodes (Pooled)	>0.05	0.007	>0.05	>0.05	>0.05	>0.05
Bone Marrow	0.04	0.01	0.01	0.007	0.02	>0.05
Blood	0.01	0.02	0.02	0.04	>0.05	>0.05
Small intestine	>0.05	>0.05	0.01	>0.05	>0.05	>0.05
Kidneys	0.02	0.0001	0.01	0.0004	0.0006	0.01
Total % Recovery	>0.05	0.002	>0.05	>0.05	>0.05	>0.05

When P > 0.05, difference is non-significant

TABLE 9

THE FILTRATION OF ^{3}H THYMIDINE LABELLED CELLS THROUGH NYLON WOOL COLUMNS

FRACTION NO.	CELL COUNT x 10^{-6}	CPM OFF COLUMN COUNTER 1*	CPM OFF COLUMN COUNTER 2**	CPM PER 10^{7} CELLS COUNTER 1	CPM PER 10^{7} CELLS COUNTER 2
1	1.74	11,626	21,270	66,816	122,241
2	18.06	99,702	173,850	55,206	96,262
3	6.78	35,337	62,650	52,119	92,404
4	0.76	5,244	9,514	69,000	125,184
5	0.2	1,759	3,328	87,950	166,400
Sample of cells put on to column	1.96	11,408	19,072	58,204	97,306

		COUNTER 1	COUNTER 2
CPM put on to column	=	285,200	476,818
CPM off column	=	153,668	270,613
% yield of input	=	53.9	56.7
Cells put on to column	=	4.7×10^{7}	
Cells off column	=	2.75×10^{7}	
% yield of input	=	58.5	

* Intertechnique β counter
** Beckman LS200B β counter

TABLE 10

THE LOCALISATION PATTERNS OF ^{51}Cr LABELLED T CELLS PRODUCED USING CYCLOPHOSPHAMIDE

Mouse source - ARC

TIME HOURS	SPLEEN	LUNGS	LIVER	LYMPH NODES	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
0.25	16.8 ± 1.94	23.2 ± 0.91	19.82 ± 0.25	2.61 ± 0.24	0.54 ± 0.03	15.83 ± 1.63	1.86 ± 0.04	2.28 ± 0.08	83.8 ± 1.03
0.5	19.63 ± 0.23	17.7 ± 0.33	20.04 ± 0.3	2.32 ± 0.27	0.55 ± 0.04	14.71 ± 0.45	1.77 ± 0.03	3.07 ± 0.03	80.8 ± 0
1	29.42 ± 0.8	9.11 ± 0.06	13.33 ± 0.16	7.06 ± 0.27	0.68 ± 0.12	4.85 ± 0.59	2.72 ± 0.53	1.65 ± 0.4	69.3 ± 0.56
2	27.69 ± 1.49	4.1 ± 0.52	12.94 ± 1.11	9.23 ± 2.35	0.86 ± 0.1	4.24 ± 0.28	2.76 ± 0.04	1.13 ± 0.06	63.2 ± 2.33
4	28.97 ± 1.47	2.44 ± 0.6	15.23 ± 1.06	7.95 ± 0.35	1.04 ± 0.13	3.36 ± 0.13	1.57 ± 0.11	1.38 ± 0.2	62.4 ± 1.91
24	19.34 ± 0.8	0.9 ± 0.06	15.75 ± 0.94	10.71 ± 0.74	0.71 ± 0.14	3.16 ± 1.17	1.94 ± 0.71	1.50 ± 0.26	54.35 ± 0.92

TABLE 11

THE LOCALISATION PATTERNS OF ^{51}Cr LABELLED T CELLS PRODUCED BY NYLON WOOL FILTRATION

Mouse source - ARC

TIME (HOURS)	SPLEEN	LUNGS	LIVER	LYMPH NODES	BONE MARROW	BLOOD	SMALL INTESTINE	KIDNEYS	TOTAL % RECOVERY
0.25	11.36 ± 1.36	36.26 ± 6.38	16.81 ± 3.09	2.07 ± 0.18	0.54 ± 0.15	7.85 ± 0.72	1.9 ± 0.34	2.79 ± 0.93	80.77 ± 5.15
0.5	18.41 ± 2.98	17.53 ± 9.0	13.53 ± 2.06	3.46 ± 0.88	1.05 ± 0.1	7.0 ± 3.52	2.37 ± 0.36	2.53 ± 0.99	66.5 ± 8.23
1	29.87 ± 4.76	6.12 ± 1.34	11.5 ± 1.09	6.97 ± 2.5	1.05 ± 0.49	4.66 ± 1.22	2.65 ± 0.99	1.76 ± 0.85	64.98 ± 8.07
2	36.93 ± 2.28	2.99 ± 0.65	9.65 ± 2.47	6.37 ± 0.65	1.32 ± 0.07	1.57 ± 0.44	2.4 ± 0.64	1.16 ± 0.61	62.2 ± 2.05
4	36.46 ± 3.32	1.81 ± 0.79	9.79 ± 2.55	9.59 ± 1.33	1.25 ± 0.44	1.43 ± 0.13	2.95 ± 1.17	1.06 ± 0.5	64.57 ± 3.15
24	18.5 ± 3.55	1.03 ± 0.27	13.54 ± 2.04	10.83 ± 1.14	0.7 ± 0.16	1.2 ± 0.23	2.32 ± 0.66	1.55 ± 0.33	49.9 ± 3.89

TABLE 12

DIFFERENCES IN LOCALISATION BETWEEN CYCLOPHOSPHAMIDE AND NYLON WOOL COLUMN PRODUCED T CELLS

Figures show P values of significance of t test. Only those organs which exhibit differences are shown - in all other organs, the differences are non-significant.

The arrows indicate increases or decreases in cyclophosphamide T cell localisation with respect to nylon wool column T cell localisation.

TIME (HOURS)	SPLEEN	LIVER	BONE MARROW	BLOOD
0.25	▲0.03	NS	NS	▲0.004
0.5	NS	▲0.01	▼0.003	▲0.04
1	NS	NS	NS	NS
2	▼0.007	NS	NS	▲0.002
4	▼0.04	NS	NS	▲0.0001
24	NS	NS	NS	▲0.004

NS = non-significant

TABLE 13

THE EFFECT OF CELL INOCULUM NUMBER ON ^{51}Cr LABELLED LYMPHOCYTE LOCALISATION

Mouse source - Bact. & Imm.

At 4 hours

Cell source	Cell Dose $\times 10^{-6}$	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery
Lymph node	3	30.53 ± 2.08	3.29 ± 0.02	10.72 ± 0.83	10.7 ± 0.83	5.94 ± 1.02	0.81 ± 0.13	3.37 ± 0.14	3.34 ± 0.35	0.84 ± 0.18	69.8 ± 1.58
Lymph node	30	30.11 ± 0.47	2.8 ± 0.35	10.81 ± 0.95	8.85 ± 1.14	6.72 ± 0.17	0.93 ± 0.03	4.03 ± 0.28	3.83 ± 0.66	0.62 ± 0.15	69.8 ± 0.11
Thymocyte	3	24.78 ± 0.55	2.67 ± 0.25	24.65 ± 2.02	1.15 ± 0.12	0.77 ± 0.08	1.2 ± 0.81	2.66 ± 0.15	0.89 ± 0.2	2.14 ± 0.1	61.4 ± 0.31
Thymocyte	35	24.5 ± 0.15	2.29 ± 0.17	21.9 ± 4.52	1.04 ± 0.08	0.69 ± 0.1	1.98 ± 0.57	3.67 ± 0.56	0.59 ± 0.03	1.98 ± 0.15	59.2 ± 3.36

No differences between the dose regimes are significant.

TABLE 14

THE EFFECT OF LARGE NUMBERS OF UNLABELLED CELLS ON ^{51}Cr LABELLED LNL

Mouse source - Bact. & Imm.

Time (Hours)	Experiment *	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	n
4	Control	26.46 ± 3.2	2.95 ± 0.68	11.97 ± 1.91	9.49 ± 1.14	6.01 ± 1.06	0.82 ± 0.23	2.58 ± 0.83	3.38 ± 0.7	1.04 ± 0.22	65.0 ± 4.0	
4	LN10x	26.15 ± 2.42	2.98 ± 0.3	12.81 ± 2.37	9.37 ± 1.37	5.72 ± 0.73	0.92 ± 0.21	2.81 ± 0.95	3.14 ± 0.37	1.03 ± 0.33	64.9 ± 1.51	
4	THY10x	32.8 ± 5.6	3.36 ± 0.44	13.04 ± 2.05	9.13 ± 1.37	6.33 ± 1.37	0.95 ± 0.17	2.1 ± 0.83	3.22 ± 0.57	1.22 ± 0.29	72.6 ± 6.9	
P value vs. control		0.002									0.003	
24	Control	16.96 ± 1.3	1.21 ± 0.53	12.06 ± 1.46	10.85 ± 1.2	7.8 ± 0.85	0.45 ± 0.06	2.0 ± 0.62	3.6 ± 1.09	0.97 ± 0.27	57.2 ± 4.27	
24	THY10x	17.85 ± 3.75	1.0 ± 0.2	10.8 ± 0.64	11.15 ± 0.84	7.06 ± 1.18	0.49 ± 0.25	1.67 ± 0.54	2.95 ± 0.24	0.87 ± 0.22	54.3 ± 3.41	

* See experimental protocols.

Only differences shown with P values are significant

TABLE 15

THE EFFECT OF LARGE NUMBERS OF UNLABELLED THYMOCYTES ON THE LOCALISATION PATTERNS OF ^{3}H URIDINE LABELLED LNL

Mouse source - B & K

Data show % recovery of input in spleen as determined by scintillation counting

	TIME (HOURS)			
	0.25	1	4	24
Control	8.36	17.88	13.6	10.0
THY 10x	9.7	19.41	18.4	13.59
$\frac{\text{THY 10x}}{\text{Control}} \times 100$	116	110	135	136

TABLE 16

THE EFFECT OF LARGE NUMBERS OF UNLABELLED THYMOCYTES ON THE INTRASPLENIC DISTRIBUTION OF ^{3}H URIDINE LABELLED LNL

Mouse source - ARC

TIME (HOURS)	EXPERIMENT	VALUES AS PERCENTAGE RECOVERY OF TOTAL				
		RED PULP	MARGINAL ZONE	PERIPHERAL WHITE PULP	THYMUS DEPENDENT AREA OF W.P.	CELLS COUNTED
0.25	Control	87.5	10.2	2.0	0.3	305
	+ THY 10x	66.9	27.6	5.5	0	326
1	Control	77.0	10.6	10.9	1.4	357
	+ THY 10x	57.3	22.1	17.1	3.5	398
24	Control	17.9	2.7	73.1	6.3	364
	+ THY 10x	20.1	1.9	75.5	2.5	319

TABLE 17

THE EFFECT OF UNLABELLED B LYMPHOCYTES ON THE LOCALISATION OF ^{51}Cr LABELLED LNL

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	n
Control	0.5	20.8 ± 3.5	22.3 ± 2.99	18.55 ± 2.33	1.97 ± 0.5	1.91 ± 0.8	0.53 ± 0.1	7.75 ± 2.65	2.25 ± 0.18	1.59 ± 0.4	78 ± 3.9	5
+ B	0.5	15.30 ± 3.96	20.24 ± 5.5	22.72 ± 6.55	2.01 ± 1.06	1.57 ± 0.76	0.58 ± 0.28	5.28 ± 1.46	2.32 ± 0.65	1.41 ± 0.36	72 ± 6.5	4
Control	4	28.83 ± 0.65	2.54 ± 0.31	15.22 ± 0.99	3.34 ± 0.58 6.59 ± 1.24	3.25 ± 0.74	0.88 ± 0.16	1.92 ± 0.21	2.95 ± 0.35	1.37 ± 0.32	60.6 ± 1.5	4
+ B	4	24.4 ± 1.98	2.29 ± 0.63	13.83 ± 0.81	4.42 ± 0.74 9.26 ± 0.38	4.83 ± 1.12	1.78 ± 0.22	0.79 ± 0.37	2.87 ± 1.89	0.95 ± 0.19	58.1 ± 2.83	2
P value vs. control		0.01			0.05		0.004	0.007				

Only those P values shown are significant

TABLE 18

THE EFFECT OF UNLABELLED B LYMPHOCYTES ON THE LOCALISATION OF ^{51}Cr LABELLED LNL

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery
Control	4	28.4 ± 1.29	2.41 ± 0.47	9.27 ± 0.58	4.79 ± 0.54	4.79 ± 1.36	1.03 ± 0.17	1.91 ± 0.22	3.40 ± 1.1	0.83 ± 0.08	57.0 ± 0.77
+ B	4	29.51 ± 2.34	2.24 ± 0.28	11.91 ± 1.88	6.33 ± 1.28	5.34 ± 0.25	0.96 ± 0.17	1.41 ± 0.36	2.90 ± 0.14	0.80 ± 0.18	61.7 ± 2.96
P value vs. control				0.04							

Only those differences with P values are significant

TABLE 19

THE EFFECT OF LARGE NUMBERS OF UNLABELLED THYMOCYTES ON ^{51}Cr LABELLED SPLEEN LYMPHOCYTE LOCALISATION

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	r
Control	4	22.2 ± 2.1	6.32 ± 0.68	28.5 ± 3.7	1.36 ± 0.44	1.72 ± 0.4	1.34 ± 0.28	3.48 ± 0.58	1.65 ± 0.15	1.58 ± 0.76	68.43 ± 4.33	4
+ THY 3x	4	23.43 ± 1.47	6.43 ± 0.42	21.17 ± 0.83	1.60 ± 0.02	2.15 ± 0.63	1.61 ± 0.38	3.79 ± 0.81	1.84 ± 0.22	2.18 ± 0.25	64.93 ± 1.79	3
P value vs. control				0.02								

Only those P values shown are significant

TABLE 20

THE EFFECT OF LARGE NUMBERS OF UNLABELLED THYMOCYTES ON THE LOCALISATION OF ^{51}Cr LABELLED SPLEEN B LYMPHOCYTES

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Pooled Lymph Nodes	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery
Control	4	22.1 ± 3.18	3.65 ± 1.5	32.1 ± 1.31	1.04 ± 0.12	1.86 ± 0.42	4.97 ± 1.1	0.7 ± 0.27	1.28 ± 0.36	68.1 ± 2.08
+ THY 5x	4	18.6 ± 2.12	4.0 ± 0.3	34.4 ± 0.92	0.59 ± 0.04	1.84 ± 0.35	3.13 ± 0.46	0.92 ± 0.05	1.42 ± 0.12	65.3 ± 3.5
P value vs. control					0.004					

Only those P values shown are significant

TABLE 21

PROPERTIES OF THYMOCYTE IMFs USED

IMF CODE	PROTEIN CONTENT (µg/ml)	ACTIVITY (units/ml x 10^{-3})
TF 120	not measured	66
TF 122	180	98
TF 123	150	400
TF 124	220	400
TF 125	100	130
TF 126	100	130
TF 127	200	3000

TABLE 22

CYTOTOXICITY OF TF 127

TIME (HOURS)	CYTOTOXIC INDEX CONTROL	IMF TREATED
0	0.19	0.08
1	5.10	4.25
2	8.79	10.31
3	10.13	13.38
4	11.34	14.03 (viability by trypan blue exclusion = 92%)

TABLE 23

THE EFFECT OF INTRAVENOUSLY INJECTED THYMOCYTE IMF

Time (Hours)	Injection	Leucocyte Count/ml $\times 10^{-6}$	% Ig Positive Lymphocytes		Blood Leucocyte Smears		n
			Peripheral Blood	Spleen	% Lymphocytes	% PML	
2	Saline	2.92 ± 0.71	34.9	55.2	35.4	63.4	10
2	IMF in saline	4.06 ± 1.00 $p = 0.009$	64.25	34.7	36.9	62.1	10
24	Saline	3.7 ± 1.13	36.8	53.7	74.3	24.6	16
24	IMF in saline	3.34 ± 0.7 $p = N.S.$	46.9	38.4	75.7	23.5	15

TABLE 24

THE EFFECT OF INTRAVENOUSLY INJECTED T IMF 122 ON THE LOCALISATION OF ^{51}Cr LABELLED LNL

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	n
Control	1	28.5 ± 0.99	9 ± 1.7	13.05 ± 0.64	3.1 ± 0.26	1.64 ± 0.14	0.87 ± 0.33	7.29 ± 0.2	2.07 ± 0.16	1.98 ± 0.37	68 ± 0.28	2
+ IMF	1	32.0 ± 2.5	10.4 ± 0.65	16 ± 2.55	1.97 ± 0.03	1.68 ± 0.33	0.65 ± 0.26	6.13 ± 1.89	2.1 ± 0.06	1.96 ± 0.04	73.3 ± 3.1	2
P value vs control					0.03							
Control	4	31.05 ± 0.39	2.59 ± 0.38	11.78 ± 0.26	5.18 ± 0.46	2.84 ± 0.38	1.52 ± 0.37	1.85 ± 0.09	2.72 ± 0.59	1.35 ± 0.15	61.7 ± 1.38	4
+ IMF	4	36.08 ± 1.85	2.93 ± 0.24	14.27 ± 0.64	4.29 ± 0.26	3.19 ± 0.35	0.85 ± 0.07	2.03 ± 0.42	2.33 ± 0.5	1.36 ± 0.16	67.67 ± 2.44	4
P value vs control		0.002		0.0004	0.015		0.013					
Control	24	17.98 ± 1.64	1.13 ± 0.36	10.36 ± 1.19	8.62 ± 1.22	9.18 ± 0.69	0.91 ± 0.04	0.87 ± 0.30	6.4 ± 1.32	1.38 ± 0.41	57.2 ± 2.14	3
+ IMF	24	19.28 ± 1.68	0.91 ± 0.18	10.30 ± 1.19	8.51 ± 0.47	9.02 ± 0.33	0.98 ± 0.08	0.98 ± 0.25	6.08 ± 0.19	1.10 ± 0.17	57.5 ± 2.88	3

Only those differences shown with P values are significant

TABLE 25

THE EFFECT OF INTRAVENOUSLY INJECTED T IMF 124 AND THYMOCYTE CONDITIONED MEDIUM ON THE LOCALISATION OF ^{51}Cr LABELLED LNL

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	n
Control	4	27.42 ± 4.4	4.97 ± 0.86	16.13 ± 1.98	6.23 ± 0.93	4.16 ± 1.14	1.06 ± 0.24	2.0 ± 0.53	2.66 ± 0.45	0.92 ± 0.34	66.01 ± 6.5	5
+ IMF	4	32.58 ± 0.89	3.91 ± 0.46	16.49 ± 0.42	4.06 ± 0.5	4.41 ± 0.33	0.97 ± 0.23	1.47 ± 0.31	2.14 ± 0.15	0.85 ± 0.11	67.3 ± 1.76	6
P value vs control		0.02	0.03		0.0008							
Conditioned* Medium	4	30.03 ± 1.73	5.61 ± 0.38	15.85 ± 0.57	5.96 ± 0.75	5.29 ± 1.13	0.87 ± 0.19	2.17 ± 034	2.62 ± 0.19	0.79 ± 0.09	69.7 ± 1.54	4

Only those differences with P values are significant

* See experimental protocols

TABLE 26

THE EFFECT OF INTRAVENOUSLY INJECTED T IMF 124 ON THE LOCALISATION OF ^{51}Cr LABELLED LNL

Mouse source - B & K

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery
Control	2	22 ± 2.07	14.93 ± 2.3	25.28 ± 2.05	1.01 ± 0.23	1.66 ± 0.22	0.76 ± 0.24	2.91 ± 0.41	1.66 ± 0.22	1.35 ± 0.16	72.1 ± 3.95
+ IMF	2	19.75 ± 2.7	12.95 ± 0.46	32.53 ± 3.8	0.58 ± 0.12	1.01 ± 0.32	0.75 ± 0.03	2.66 ± 0.54	1.64 ± 0.36	1.76 ± 0.18	74.3 ± 0.94
P value vs control				0.04	0.05	0.044					
Control	8	18.87 ± 0.77	5.1 ± 1.08	23.97 ± 0.26	2.01 ± 0.71	3.16 ± 0.27	0.71 ± 0.11	1.28 ± 0.13	1.55 ± 0.04	1.13 ± 0.07	59.13 ± 0.08
+ IMF	8	18.15 ± 1.67	5.93 ± 2	32.89 ± 0.5	1.22 ± 0.24	2.69 ± 0.57	0.63 ± 0.06	1.67 ± 0.88	1.38 ± 0.28	1.59 ± 0.26	66.65 ± 3.35
P value vs control				0.0001							

Only those differences shown with P values are significant.

TABLE 27

THE EFFECT OF THYMOCYTE IMF 124 ON THE INTRASPLENIC DISTRIBUTION OF LABELLED LNL

Mouse source - ARC

LNL labelled with ^{3}H uridine. Treated with 25,000 units of T IMF 124

Time (Hours)	Experiment	Red Pulp	Marginal Zone	Peripheral White Pulp	Thymus dependent area of white pulp	Cells counte
0.25	Control	87.5	10.2	2.0	0.3	305
0.25	+ IMF	49.5	40.8	9.7	0	319
1	Control	77.0	10.6	10.9	1.4	357
1	+ IMF	63.1	21.6	12.8	2.5	320
24	Control	17.9	2.7	73.1	6.3	364
24	+ IMF	17.5	2.3	72.8	7.4	309

TABLE 28

THE EFFECTS OF INTRAVENOUS THYMOCYTE IMF 126 AND THE PRETREATMENT OF CELLS WITH THYMOCYTE IMF 126 ON THE LOCALISATION PATTERNS OF ^{51}CHROMIUM LABELLED LNL

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Total % Recovery	n
Control	4	28.53 ± 4.31	11.32 ± 0.42	5.30 ± 1.5	3.76 ± 0.51	1.6 ± 0.27	1.71 ± 0.55	3.50 ± 0.69	60.22 ± 3.83	7
+ IMF	4	28.83 ± 2.34	14.17 ± 0.88	2.83 ± 0.36	2.61 ± 0.24	1.56 ± 0.18	1.22 ± 0.38	2.05 ± 0.21	57.3 ± 2.16	5
P value vs. control			0.00001	0.005	0.0009			0.001		
+ IMF + wash	4	31.94 ± 2.1	13.01 ± 1.05	3.13 ± 0.84	2.31 ± 0.39	1.26 ± 0.22	1.48 ± 0.52	1.90 ± 0.25	59.43 ± 4.11	7
P value vs. control			0.002	0.006	0.0001			0.0001		

TABLE 29

THE EFFECT OF T IMF 122 ON THE LOCALISATION OF ^{51}Cr LABELLED SPLEEN B LYMPHOCYTES

Mouse source - ARC

Experiment	Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery
Control	2	24.5 ± 1.13	5 ± 0.14	33.2 ± 2.8	0.60 ± 0.2	0.37 ± 0.07	1.2 ± 0.13	5.6 ± 0.85	0.59 ± 0.01	1.17 ± 0.09	72.6 ± 2.76
+ IMF	2	26.5 ± 2.83	6 ± 0.42	41.75 ± 0.07	0.46 ± 0.03	0.35 ± 0.11	1.4 ± 0	5.59 ± 0.56	0.55 ± 0.08	1.22 ± 0.35	84.88 ± 2.57
P value vs. control				0.05							
Control	4	22.1 ± 3.18	3.65 ± 1.5	32.1 ± 1.31	0.63 ± 0.08	0.41 ± 0.07	1.86 ± 0.42	4.97 ± 1.1	0.7 ± 0.27	1.28 ± 0.36	68.1 ± 2.08
+ IMF	4	20.7 ± 4.9	3.67 ± 1.4	38.4 ± 8.34	0.44 ± 0.1	0.27 ± 0.05	1.56 ± 0.31	4.16 ± 0.68	0.72 ± 0.12	1.43 ± 0.48	72.4 ± 13.4
P value vs. control					0.01	0.01					

Only those differences shown with P values are significant.

TABLE 30

COMPARISON OF THE EFFECTS OF VARIOUS THYMOCYTE IMFs ON LNL LOCALISATION AFTER 4 HOURS

IMF CODE	SPLEEN	LUNGS	LIVER	LYMPH NODES PERIPHERAL	LYMPH NODES MESENTERIC	BONE MARROW	BLOOD	SMALL INTESTINE	TOTAL % RECOVERY
122	▲*	-	▲*	▼*	-	▼*	-	▼?	▲
124	▲*	▼*	-	▼*	-	-	▼	▼?	-
126	-	-	▲*	▼*	▼*	-	▼	▼*	-

Legend: ▲▼ slight, non-significant increase (decrease) over control value.

▲*, ▼* significant increase (decrease) over control value.

\- no difference between IMF treatment and control.

TABLE 31

THE LOCALISATION PATTERNS OF ^{51}Cr LABELLED LYMPH NODE B LYMPHOCYTES PRODUCED BY IN VIVO ALS TREATMENT

Mouse source - ARC

Time (Hours)	Spleen	Lungs	Liver	Lymph Nodes Peripheral	Lymph Nodes Mesenteric	Bone Marrow	Blood	Small Intestine	Kidneys	Total % Recovery	n
4	19.29 ± 5.66	4.50 ± 3.32	38.31 ± 6.8	0.12 ± 0.05	0.16 ± 0.07	1.27 ± 0.53	4.77 ± 1.75	0.65 ± 0.08	2.37 ± 1.50	71.9 ± 5.97	8

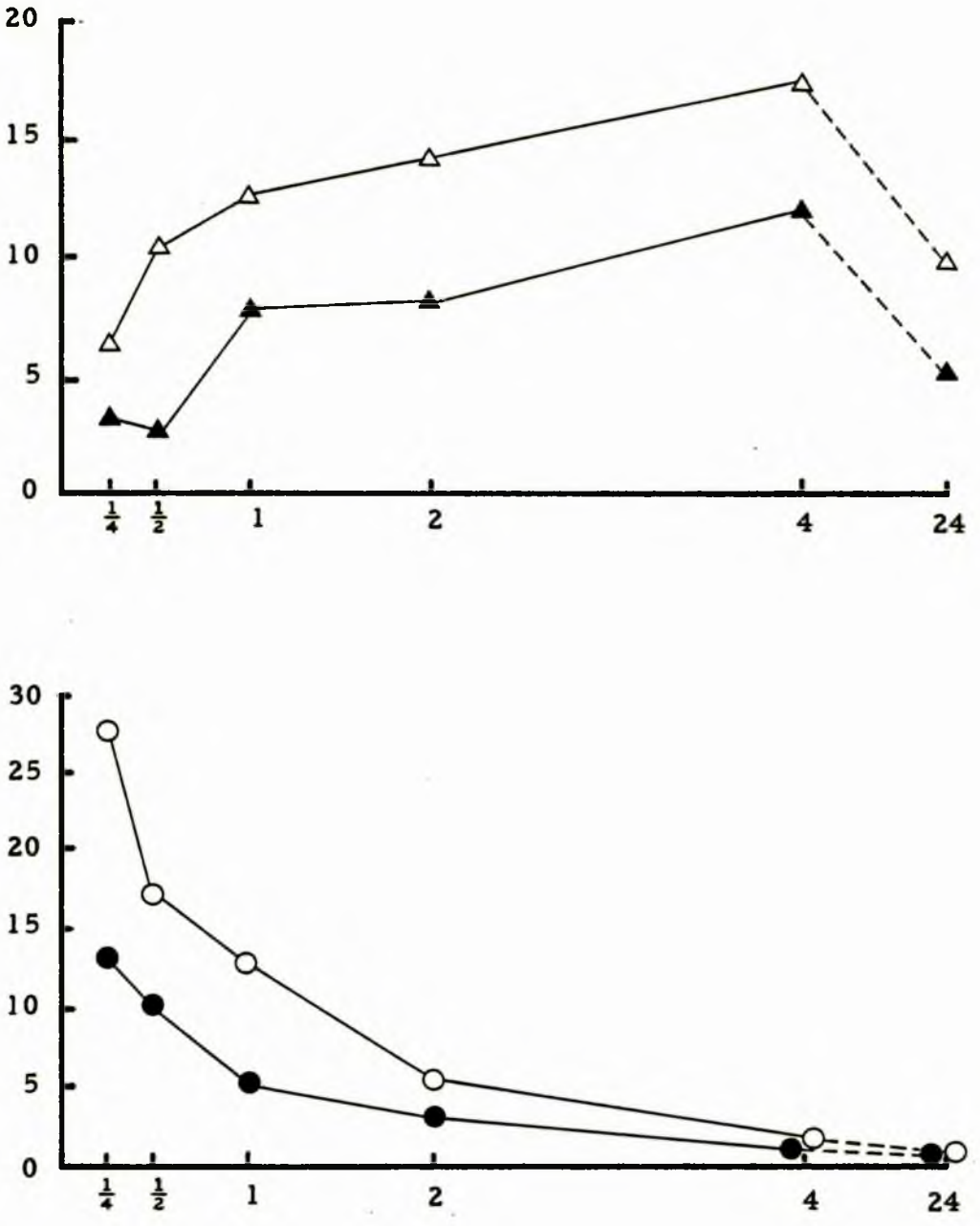


Figure 1a Time course of recoverable radioactivity: abscissae - time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.

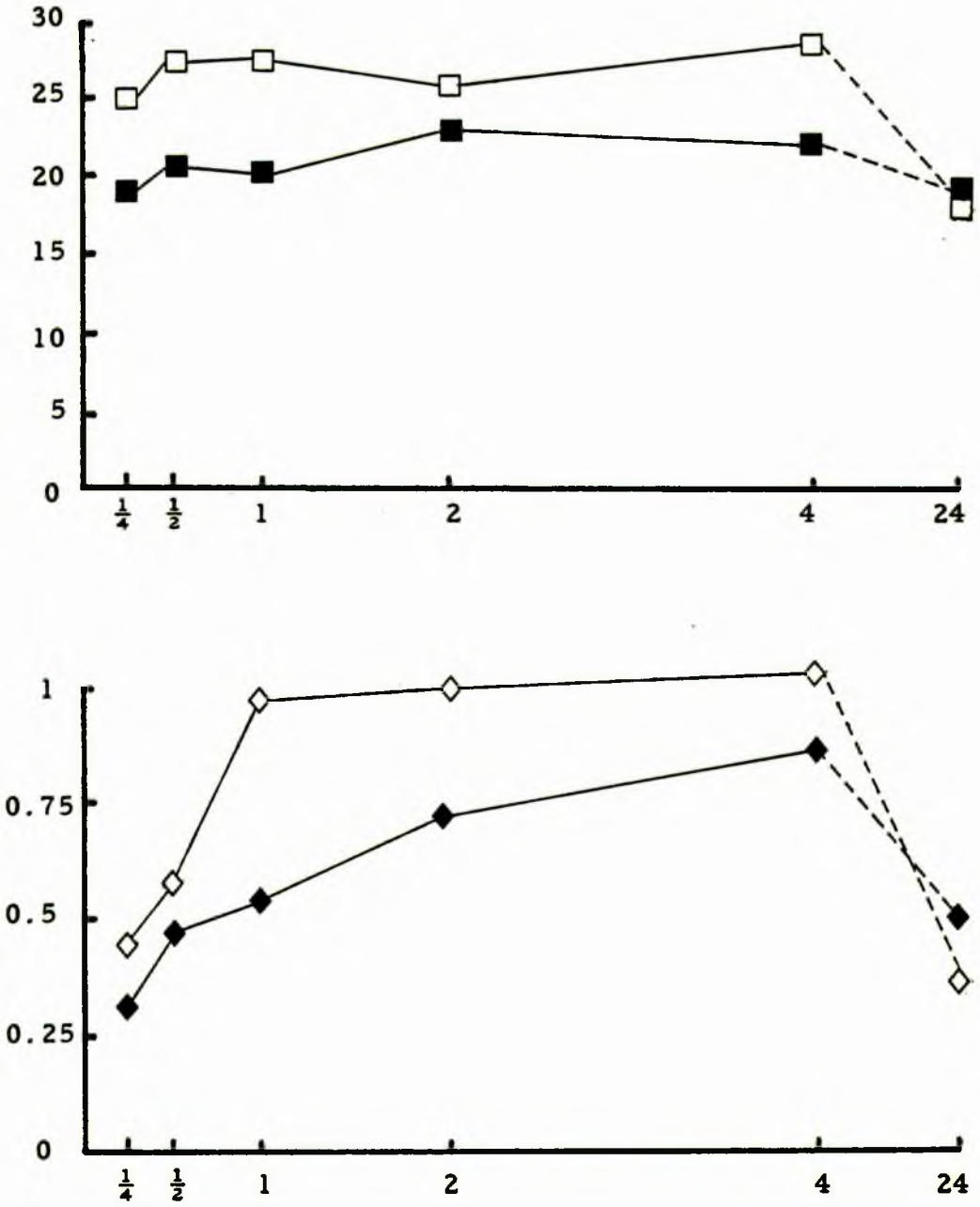


Figure 1b Time course of recoverable radioactivity: abscissae - time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.

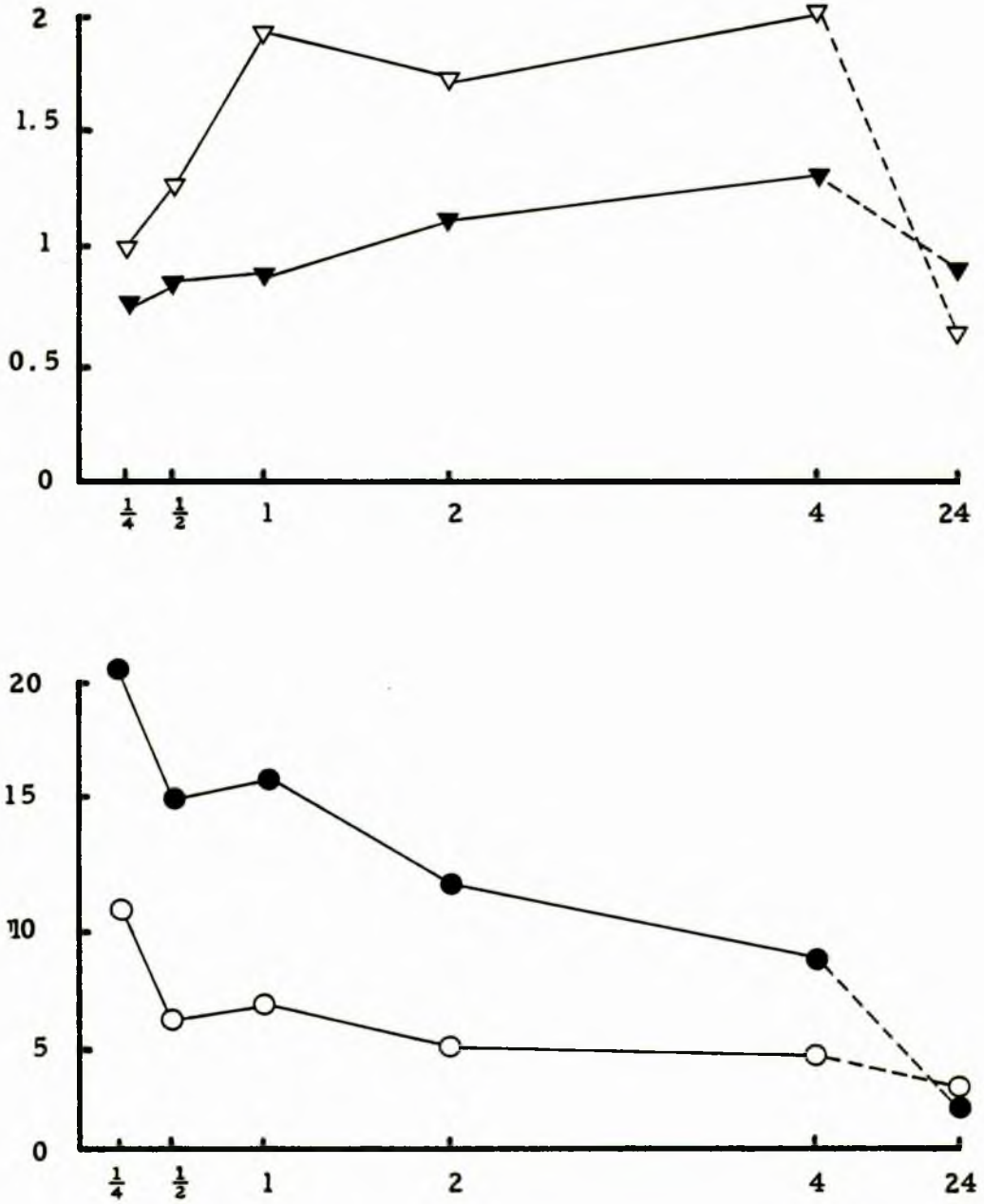
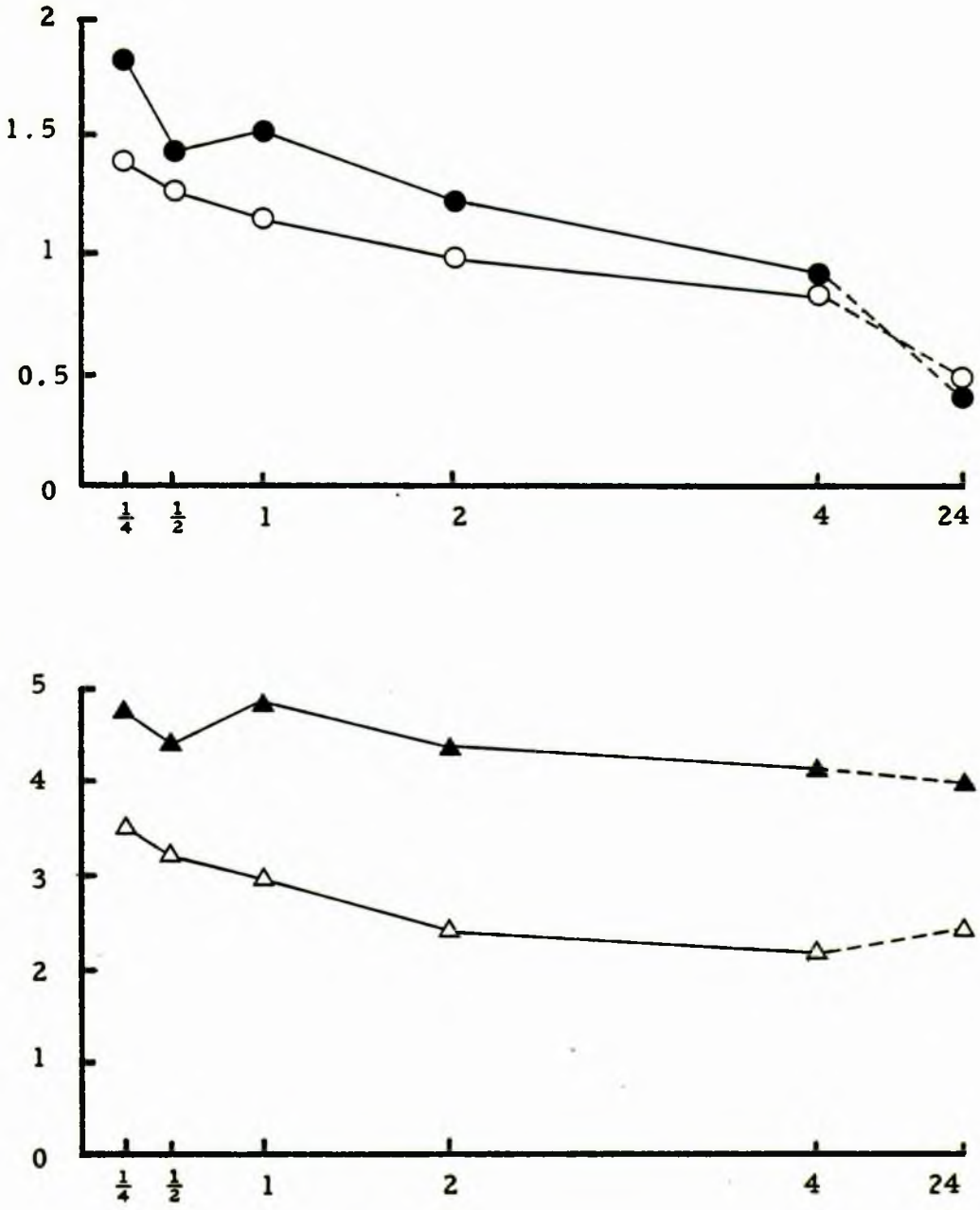


Figure 1c Time course of recoverable radioactivity: abscissae time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.



○● Small intestine

△▲ Kidneys

Open Symbols - whole thymus population

Solid Symbols - filtered thymus subpopulation

Figure 1d Time course of recoverable radioactivity: abscissae - time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.

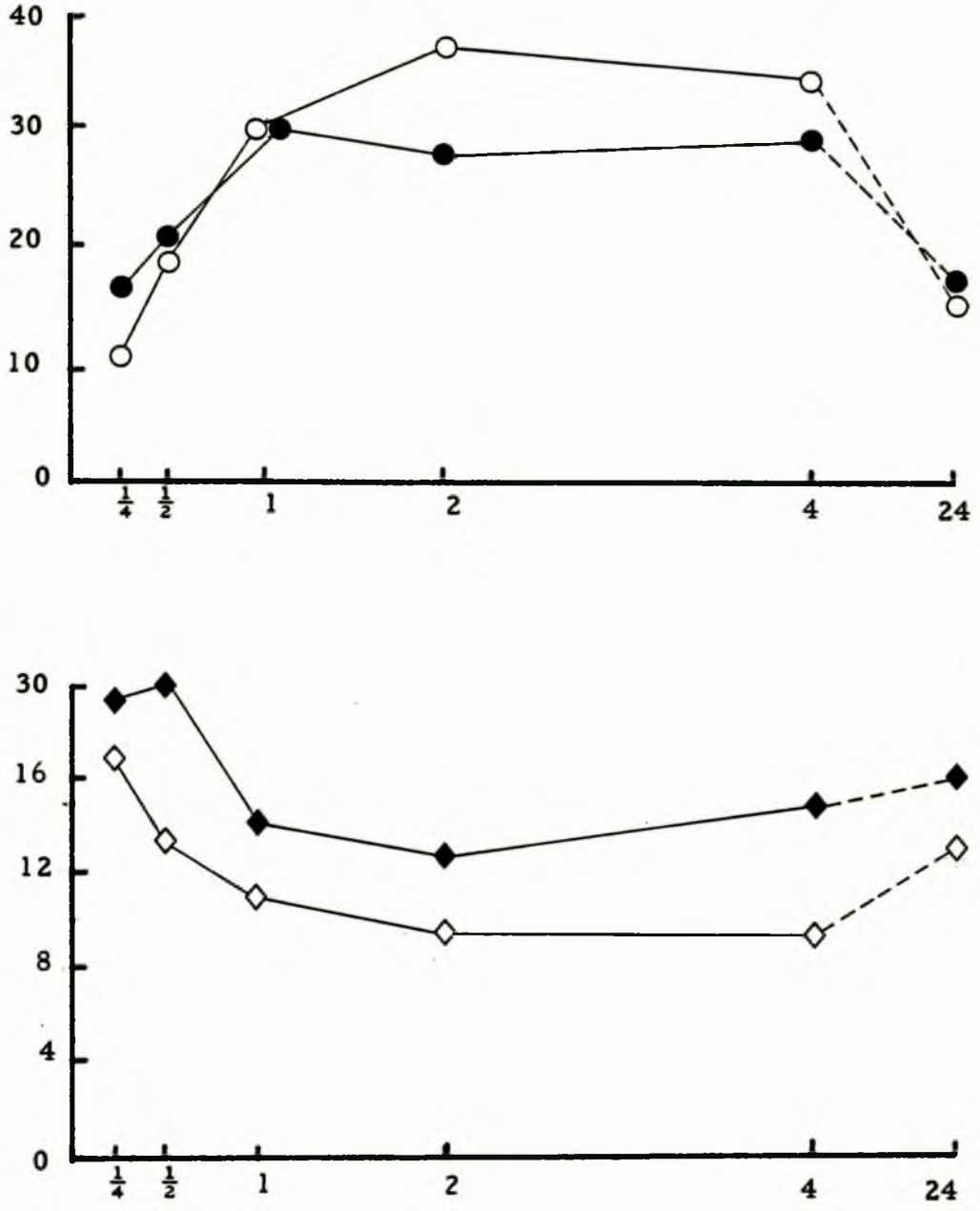


Figure 2a Time course of recoverable radioactivity: abscissae - time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.

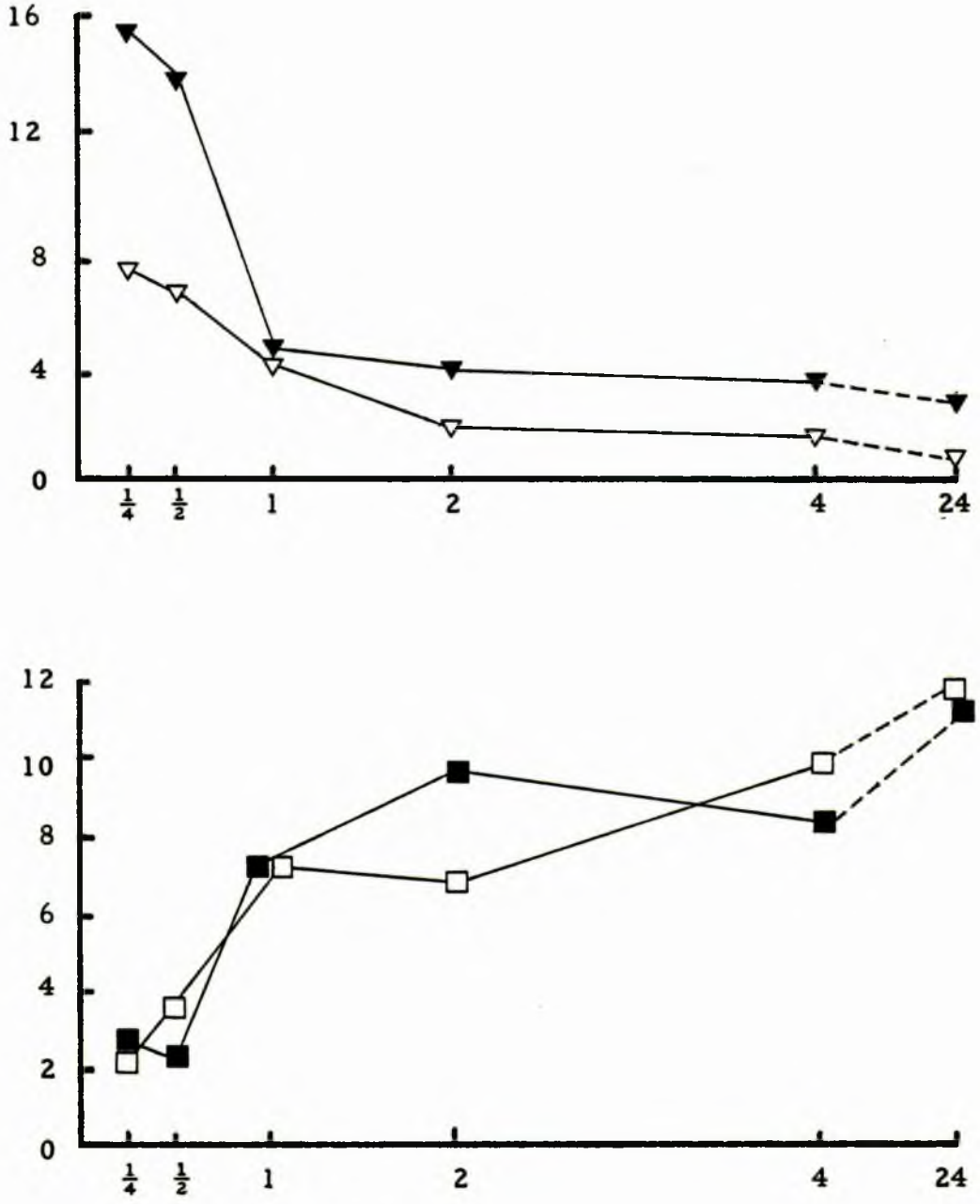


Figure 2b Time course of recoverable radioactivity: abscissae - time in hours after injection of labelled cells; ordinates - recovery as a percentage of the injected dose.

CHAPTER SEVEN

DISCUSSION

CHAPTER SEVEN

DISCUSSION

In this chapter, the following points will be discussed:

1. The experimental system of cell localisation used here, in particular, the uses of various radioactive labels and the characteristics of localisation in the organs sampled.

2. The correlation between the adhesiveness of cells as measured in vitro and their in vivo localisation properties utilising subpopulations of differing adhesiveness produced by nylon wool filtration.

3. The effects of large numbers of unlabelled lymphocytes of a single lineage on the localisation patterns of labelled populations of lymphocytes.

4. Products of the thymus and a comparison with thymocyte interaction modulation factor.

5. The effects of thymocyte IMFs in vivo - particularly the mobilisation of cells from the spleen and perturbations caused to the localisation patterns of circulating cells. The similarity between the effects of thymocyte IMFs and large doses of unlabelled cells of a single lineage will be discussed in detail.

6. The wider implications of the IMF theory will be suggested along with some speculation about certain mechanisms occuring in vivo which might be explained by IMFs. The IMF theory

will be compared to other theories of sorting-out and cell adhesion which have been suggested in the past for other systems. Finally, some thoughts for future experiments will be considered.

7.1 The validity and problems of using ^{51}Cr as a label for transfusion studies

Radioactive sodium chromate has been used as a label for erythrocytes in clinical work for many years but only for the past fifteen years has it been used as a routine label for lymphocytes in transfusion studies. As with most labels, the use of ^{51}chromate has had its critics and it is proposed here to briefly list the pros and cons of its use.

The dose used for labelling the cells during these experiments has been 50μCi/10^8 cells which seems to be the same as used by all other workers. However, Bainbridge & Gowland (1966) used doses up to 400μCi/10^8 cells without any apparent detrimental effect on the cells' localising ability, although Rolstad & Toogood (1978) report that doses over 10μCi/ml affect the Graft-versus-host response of rat lymphocytes.

Sodium chromate is also an oxidising agent and as was pointed out in the introduction, oxidising agents such as sodium periodate can disrupt lymphocyte localisation (Zatz et al 1972b). In this particular experiment, the effect of periodate was measured on cells labelled with ^{51}chromate so there was no indication of any effect due to the chromate alone. To check for any possible effects, [^{3}H] uridine labelled thymocytes were treated with non-radioactive chromate and their localisation patterns compared to non-treated controls. The results in table 1 indicate that chromate has little effect on lymphocytes as

far as their ability to localise in lymphoid and non-lymphoid organs is concerned. The variability of the results might mask any differences in the small intestine and kidneys, this being due to preparation techniques required for liquid scintillation counting. The main effect of periodate treatment is to reduce the localisation in the spleen and lymph nodes, cells localising preferentially in the liver (Zatz et al 1972b). This tendency cannot be seen with chromate treated cells so it is concluded that chromate labelling at the dose used in these experiments does not adversely affect any cell surface component.

The number of labels that have been used in lymphocyte transfusion studies is now large - van Rooijen (1977) lists twelve isotopic species that can be used, while Rannie & Donald (1977) have made a systematic study of six isotppic labels in common use. Their conclusion is that ^{51}chromate is probably the best all round label for its ease of use and the fact that any label eluted is not reutilised. However, Sprent (1976) suggested that eluted label was trapped in certain organs increasing the apparent recoveries. Since ^{51}Cr labels all cell types, though not necessarily evenly, great care must be taken to eliminate contaminant cells from lymphocyte suspensions.

Recently, ^{99m}Tc (Barth & Singla 1975), ^{75}Se L-selenomethionine (Rose & Micklem 1976) and ^{111}In (Rannie et al 1977b) have all been suggested as being superior to ^{51}chromate but short half lives, toxicity and difficulty of use make them more impractical. ^{51}chromate is thought to bind covalently to amino groups of lysine and other basic amino acids and elution from the cell is as a result of turnover of labelled protein and the loss of non-reutilisable labelled protein fragments

(Ronai 1969). The rate of elution of labelled fragments into the urine of humans is approximately 3% of the injected dose over the first 24 hours after infusion (Hersey 1971), although Sanderson (1964) has found that 5% of ^{51}chromate label is lost within 2.5 hours from mouse lymph node lymphocytes in culture. However, these particular cells do not maintain their viability well in culture even in complete media. The reutilisation of free injected chromate is shown on table 4, although only injected intraperitoneally and subcutaneously. Most free chromate remains in the blood, although with the intraperitoneal injections this may be an experimental artefact. However, the similar recoveries in the blood after subcutaneous injection do not support this. Since some of this ^{51}chromate injected is in the form of free label, it is hardly surprising that the blood would display the highest recovery. The liver levels may be the result of trapping of labelled protein fragments or the binding of free ^{51}chromate.

As mentioned, the presence of contaminating cells in lymphocyte suspensions is of importance with chromate labelling. In the case of the experiments reported here, erythrocytes are the major contaminant and table 2 shows the localisation of labelled erythrocytes 15 minutes and 4 hours after injection. This gives an idea of the blood content of the various organs and even if a contamination level in lymphocyte suspensions of 1% is assumed, probably a reasonable estimate, then none of the sampled organs would exhibit more than 0.5% of the total injected dose as a contaminating erythrocyte recovery. In particular, the recoveries in the spleen and lymph nodes are especially low. However, since the same population of labelled cells is always

used in control and experimental conditions in each experiment, then any differences seen are probably real and not due to contaminating cells.

An important feature of lymphocyte localisation is the recovery of labelled cells within the liver. Many reports suggest that dead cells accumulate in the liver (e.g. Wiig 1976), some suggest that they do not (Bainbridge et al 1966) which is consistent with the data presented in table 3. The results show the effect of heat-killing and glutaraldehyde fixation on the localisation patterns of labelled thymocytes. Heat-killing, not surprisingly, causes many cells to become trapped in the lungs, possibly as a result of nucleic acid leakage making the cells "sticky". Remaining cells are usually trapped in the liver. However, glutaraldehyde fixed cells, which are also effectively dead but probably not so "leaky", are not found to such a great extent in the liver, indeed, less than normal cells although the total recovery is less than with heat-killed cells. The problem of total recovery will be discussed shortly. Thus, it is not clear whether dead cells do localise in the liver and this point will again be raised in discussion of particular results where the liver levels are increased.

Cells, once outside the blood-lymph circulation, are thought to be unable to return to circulation (Hollingsworth & Carr 1972). This point is reinforced by the findings shown on table 5 of the localisation of labelled thymocytes injected intraperitoneally and subcutaneously. From neither route do the cells return to circulation to any great extent. In this case, the blood levels after intraperitoneal injection are an

artefact due to the collection technique. Injection of cells subcutaneously is thought to damage them which may account for the increased blood recovery.

The use of ^{51}chromate as a lymphocyte label, over the times used in these experiments, is considered valid and the intravenous route essential. Since the contamination of injected lymphoid cells by other cell types was always minimal, that particular criticism can be discounted. The reutilisation of ^{51}chromate released is difficult to evaluate and the results in table 4 provide little further insight. However, over the time course used in most of these experiments, routinely 4 hours, it is thought that the amount of label released which can be reutilised is minimal, provided that the cells are adequately washed prior to injection.

7.2 General notes on the organs sampled

The percentage recovery of injected dose in the organs listed in experiments here is often dependent on the sampling technique and allowance must be made for the fact that not all of a particular organ may be sampled. The notes below bring to light differences that may be apparent. Some workers have expressed the organ recovery as a percentage of the total recovery rather than as a percentage of the injected dose. This irons out variations due to differences in total recovery but can mask the effect of localisation in organs not sampled. Although there are cases where this method of expression is of use, it is not used here.

Spleen. The spleen was sampled whole after the animal was killed by exsanguination. This technique could reduce the number of cells in the red pulp by a flushing out following the rapid fluid loss but examination of the spleen histologically in animals killed by exsanguination and by cervical dislocation did not reveal any differences in cellularity of the red or white pulp. About 60% of splenic lymphocytes are thought to be of the recirculating pool (Ford 1969).

Lungs. The amount of blood remaining in the lungs after exsanguination varied depending on the efficiency of blood removal. The presence of cells in the lungs also needs explanation. Following intravenous injection of cells, the lungs are the first capillary bed to be encountered and thus there is often a transient hold-up before cells are released, as can be seen from the data in table 7. It has been suggested that surface changes brought on by in vitro handling might cause a hold-up in the lungs which will subsequently affect the availability of cells to other organs like the lymph nodes and spleen (Bradfield & Born 1973). Slow injection of cells intravenously is thought to help reduce lung levels but this was not found to be altogether true. In an early study, Weisberger et al (1951) suggested that the lungs might act as a homeostatic organ in maintaining blood leucocyte levels, an idea which seems to have little support. Also of interest is the bronchial lymphoid tissue found in the bronchial mucosa, which, although morphologically similar to Peyer's patches, shows no features common to peripheral lymphoid tissue (Bienenstock et al 1973). The bronchoalveolar lymphocytes are mainly T cells and may be present in response to (or to respond to) antigen entering the respiratory tract (Danielle

et al 1977). Whether transfused lymphocytes are held-up in the lungs due to some interaction with bronchio-lymphoid tissue or purely by physical constraints is not known and is of interest in the future, especially from the point of view of immunisation by aerosols.

Liver. As was mentioned earlier, the hepatic localisation of lymphocytes has been thought to be due in part to dead cells, but from the data here, this may not be entirely true. The localisation of labelled lymphocytes in the liver depends on the lineage of the injected cell - 10-15% of injected T cells are found in the liver after 4 hours (tables 10 & 11) whereas 35% of injected B cells localise in the liver (tables 20 & 31). Clearly, the liver cells interact with some surface receptor on B cells, as has been discussed in the introduction, with respect to the effect of neuraminidase on transfused cells. From the data in tables 10 & 11 (which is typical), it can be seen that the liver level of injected cells reaches a maximum after 15 minutes (or possibly quicker), which does not increase, and in fact decreases slightly with time. This is not consistent with cells being removed after dying in circulation or the immediate sequestration of dead or moribund cells following infusion, since the levels are peculiar to the particular cell type. Is there then a resident population of cells within the liver? Chronic irradiation of the liver has no effect on recirculating lymphocytes (Ford 1968) so if there is a population which is resident then it is probably of a non-recirculating type.

Lymph nodes. In most of the experiments, the lymph nodes were assayed as two different types. Peripheral nodes - inguinal, brachial and axillary; and mesenteric nodes. In sheep, it seems that

recirculating T cells from intestinal lymph home particularly back to the intestine and not as well to lymph nodes and vice versa, possibly since intestinal lymph T cells cannot cross lymph node HEVs as well as lymph node lymph T cells (Cahill et al 1977). In the mouse, however, there is no difference in the localisation patterns of mesenteric or peripheral T cells unlike that found for mesenteric and peripheral T lymphoblasts (de Freitas et al 1977). Since these two separate sets of data indicate major differences, it is interesting to see if the localisation to peripheral and mesenteric nodes is equally affected by various treatments. As will be indicated later, this has not been found to be true.

The mesenteric and inguinal nodes have been estimated to represent one third and one half of the total lymph node mass by Martin (1969) and Freitas & de Sousa (1975) respectively. In this case, the nodes sampled in this study must represent half to three quarters of the total lymph node mass, although none of the data shown here for lymph node recoveries is corrected to allow for this estimation.

De Sousa (1976) states that spleen and lymph node recovery data for the same labelled inoculum are so constant that results from different laboratories can be pooled. This may hold true for data obtained for a 24 hour time course, but in this study, using mice from different sources and shorter time intervals, the spleen and lymph node recoveries were found to be very variable. For instance, compare the four hour control data on tables 14, 17, 18 and 25 - the spleen to pooled lymph node ratios are respectively 1.71, 4.38, 2.96, 3.87 and

at 24 hours from tables 14 and 25, the ratios are 0.91 and 1.01 (it should be remembered that there are rather more lymph nodes sampled in this study). Freitas & de Sousa (1976a) give the 24 hour spleen to lymph node ratio varying between 1.06 and 2.25 for the CBA mouse. Other workers have published data which fits between these two values and is occasionally lower. De Sousa (1976) actually suggests a value of 1.5 as a mean from pooled data from five sources (two of which are from the same laboratory, using the same animals). In this instance, pooling data from different experiments must be done with great care, and only when an analysis of variance permits. For this reason, only the data for the four hour control in table 14, pooled from 18 animals, fitted the criteria and hence the remaining data is usually that pooled from several animals in a single experiment or at least identical repeats on consecutive days. The source of variation between animals from different suppliers is unknown, although mice from one supplier always displayed larger lymph nodes and a subsequently greater lymph node recovery of injected cells.

Bone marrow. To sample the bone marrow, both the femurs were removed and counted whole after the removal of adherent tissue. Both femurs in the mouse account for about 6% of the total active bone marrow (Taketa et al 1970) so to estimate the total localisation of injected lymphocytes in the bone marrow, the results should be multiplied by 16.7. Martin (1967) suggests that the recovery should be 12.5 times that for one femur. This indicates a substantial localisation of cells in many cases, e.g. about 20% of the injected dose at four hours for T cells (tables 10 & 11), a significant quantity that has often been

disregarded in some studies. The bone marrow also contains a significant amount of blood, some 18% of the total blood volume as estimated at 4 hours from table 2 and there is also some interchange between the blood and bone marrow itself (Hudson & Yoffey 1966). This may represent a normal route of recirculation of cells since some of the T cells in the marrow have been found to be part of the recirculating pool in humans (Abdou et al 1976) and lymphocytes have been seen traversing the sinusoidal endothelium between blood vessels and the marrow matrix in guinea pigs (Hudson & Yoffey 1966).

Blood. Blood was sampled by obtaining as much as possible from aortic puncture at a point below the diaphragm. Knowing the volume of blood removed and the weight of the animal before death, the actual blood recovery of labelled cells was estimated assuming that the blood volume of a mouse is 0.121ml per gram body weight (Kaliss & Pressman 1951). Barth & Singla (1975) suggest removing 0.1ml of blood and multiplying the subsequent recovery by 24 to give an estimation of the total recovery in the blood but this does not take into account the weights of experimental animals which do vary, even in the same batch. Martin (1969) suggests the blood volume to be 0.06ml per gram body weight. Which of these estimates is the most accurate is difficult to evaluate - the recoveries calculated by Kaliss & Pressman's and Barth & Singla's estimates give similar results, from Martin's estimate, the recovery is very much less, probably near a half. Since volumes up to 0.7ml can be easily removed from an 18 gram mouse (the normal weight of animals in these experiments), Martin's estimate is probably slightly low. The blood was counted whole and not separated into

lymphocytes, other leucocytes and plasma. The blood was the only organ that is expressed as a corrected total recovery in the results.

Small intestine. The whole of the small intestine, including Peyer's patches, was assayed as one organ. Thus, the results will include localisation in the lamina propria of the intestine as well. The reason for not removing the Peyer's patches from the intestine was technical; for the numbers of animals used, it would have taken too long to separate the parts.

Kidneys. Both kidneys were removed as a further assay of non-lymphoid tissue. The recovery is usually of the order of 1-3%. Weisberger et al (1951) and Laissue et al (1976) have suggested that kidney levels may be explained by the accumulation of label after destruction of the cells which may be true, except that kidney levels remain very constant with time (tables 7, 10, 11). The blood content of this particular organ is of importance but that alone cannot explain the relatively high recoveries.

Skin. In several experiments, a piece of skin 1cm^2 was assayed for radioactivity. The recovery never exceeded 0.05% of the injected dose (data not included). This is in agreement with the data of Rannie & Donald (1977) although, in their results, the migration to non-lymphoid organs like skin and muscle never exceeded 1% of the injected dose.

Thymus. In many of the earlier experiments reported here, the thymus was also assayed for radioactivity. Recovery never exceeded

0.2% of the injected dose of labelled thymocytes as against 1% with labelled thoracic duct lymphocytes (Rannie & Donald 1977). Kadish & Basch (1977) have found a subpopulation of thymocytes which is able to home back to the thymus after intravenous injection but despite this, subsequent sampling of the thymus was not continued.

Tail. In all experiments, the tail was assayed for radioactivity. Intravenous injections in mice can be slightly difficult in that the needle is of a similar diameter to the caudal vein. If the needle punctures the vein and radioactively labelled cells enter the tail itself, then the subsequent localisation results will be altered. If the injection is done correctly, then the tail recovery never exceeds 1%, and is not usually even this high. Any animal showing a tail recovery in excess of 1% was not used as a source of data. This precaution appeared to be necessary, since it was impossible to guarantee a perfect injection each time.

Total recovery. In all the data shown, the total recovery of injected cells in the organs sampled is given. This value depends on the time after injection of sampling and the cell type but as a rule, the total recovery is around 60% at 4 hours. Even at 15 minutes, only a maximum of 80% of the injected dose can be accounted for (table 10). It is important to be able to attribute the remaining 20%. Using the data on table 7, at 15 minutes, taking into account the bone marrow and lymph node weightings, the recovery is about 90% for whole thymus cells and a similar value at 4 hours. Routinely, 80-90% of the injected dose can be accounted for. The site of the remaining 10% is difficult to locate.

The major organs not sampled are the brain, heart, stomach and large intestine, although these seem to be unlikely candidates. The excretion of unutilised label after 15 minutes is minimal since counts in the cages used were not detected. In some experiments, using the same cell types for both control and treated conditions, the total recovery was seen to increase significantly, e.g. table 14. Where the increase comes from is a mystery as is the site of the unaccounted for 10%. Further suggestions will be put forward in discussions on individual experiments.

7.3 The effect of filtration through nylon wool on lymphocyte adhesiveness and localisation patterns

This work has already been published in a brief report (Evans & Davies 1977). The effect of nylon wool filtration on cell adhesiveness - filtration through nylon wool results in a subpopulation with a greater mutual adhesiveness than the input population (table 6). The value for the adhesiveness of normal thymocytes conflicts with that given by Curtis & de Sousa (1975) but in the former case, the measurements were made in the presence of serum which reduces adhesiveness. There are several possibilities for the separation of adhesive populations:

1. A more adhesive subpopulation is selected, from the entire population, which is more readily removed from the nylon wool column. The remaining cells are more adhesive to nylon wool than the eluted population but have a low mutual adhesiveness, such that the average adhesiveness of nylon wool adherent and non-adherent cells is the same as the entire thymocyte population before filtration.

2. Filtration through nylon wool makes the cells more adhesive by some unknown mechanism; the yield of the input is only about 50% since the remaining cells are physically trapped on the column.

3. Filtration through the column removes some substance which causes the cells to adhere to each other.

The second possibility seems unlikely in that the yield would be very much more variable than has been found and the trapped cells would be easy to dislodge which is known not to be true. This concept is also not reconcilable with the fact that nylon wool columns separate T and B lymphocytes from mixed cell suspensions (Greaves & Brown 1974). As yet, there is no evidence in favour of the third suggestion, although the possibility cannot be ruled out. In this case, it is suggested that the column "selects" a population of greater mutual adhesiveness. The constituency of the subpopulation is dependent upon the number of cells which are normally adherent to nylon wool but which, for some reason, are physically washed off during elution. Conversely, some cells may be trapped and not appear in the eluate when expected. The variability of the eluate from the column is reflected in the large standard deviation of the data for the adhesiveness of cells in the subpopulation (table 6) but at all times this adhesiveness was always greater than that of the input population.

With regard to the actual adhesiveness of the cells, imagine in the simplest instance two cell types in a mixture - A & B. If homotypic (specific) adhesions, i.e. A-A or B-B, are "preferred" to heterotypic,

A-B, then the average adhesiveness value for cells in this mixture (not distinguishing between cell types and assuming a roughly 50:50 mixture) will be low. If one cell type is removed, then the average overall adhesiveness will be high. Thus, cells which show high mutual adhesion show low adhesion to other cell types, a concept which is important and will be discussed further. This very simple case could apply to the thymocyte situation but is unlikely - more than two cell types are probably involved. However, if there are, for instance, four cell types, then the average overall adhesion of a mixture will be very low, if again homotypic adhesions are preferred, but if one or two of the cell types are removed or selectively retained by the column, then the overall adhesiveness will be much greater.

It is interesting to note that in one of the original papers on nylon wool filtration, Bianco et al (1970) had a much higher recovery of filtered thymocytes than reported here and concluded that nylon wool separation did not produce a subpopulation of thymocytes in the same way as it did for lymph node and spleen lymphocytes.

Having found that the separate populations differ in their mutual adhesiveness, it was decided to see whether any variations in localisation patterns could also be seen. Overall, during the first four hours after injection, there is a significantly decreased localisation of cells in most organs sampled with an accompanying increase in the blood and kidneys (see tables 7, 8, figure 1). This suggests that the more adhesive cells are less able to enter organs and remain in venous circulation, but some of the other results are at least worthy of comment. It will

be noted that the more adhesive cells localise less well in the lungs, especially at early times when the difference in localisation compared to normal thymocytes is very large. This result is, in some ways, surprising, since one might expect the more adhesive cells to become trapped as aggregates in the lung capillary beds and thus show an increased "localisation" in that organ. However, this is perhaps a simplistic point of view since after interaction with serum proteins, the adhesiveness of the cells will probably change (Curtis & Greaves 1965) - e.g. the difference in the adhesiveness of thymocytes measured in medium with and without foetal calf serum, as already mentioned. But, are the more mutually adhesive cells less adhesive to other cell types such as endothelium? This might explain all the findings except for the persistent increase in localisation in the kidneys. This feature is hard to explain from the aspect of the blood content of the organ as the localisation is still significantly raised at 24 hours and is rather more suggestive of the binding of released radioactive label. In the absence of autoradiographs of kidney sections, this problem remains unsolved. Another interesting feature is the kinetics of the localisation patterns which are easily visualised in figure 1. As was mentioned in an earlier discussion, the constancy of the liver localisation shown in figure 1 is an interesting point to note. The rate of entry of cells of the more adhesive type into the spleen, lymph nodes and bone marrow (remembering that the corrected bone marrow results make this even more significant) is also markedly depressed over the first hour, and at 24 hours both cell types have localised equally well in all organs. This suggests that one or other or both cell types have

been modified by various interactions in vivo or some ageing process which has rendered them similar. It would be interesting to measure the adhesiveness of both cell types after 24 hours but this is not practical except by incubating the separate populations in mouse serum in vitro and then making subsequent measurements. Further analysis of the cells which remain adherent to the nylon wool column would prove useful although they are so difficult to retrieve. If these cells, which are less mutually adhesive (by definition), become trapped to a greater extent in organs than normal cells, then the hypothesis suggested might have more support. However, at this time, this is in the realms of speculation.

Obviously the most important question is what the cells in the eluted subpopulation are. Are they a specific subpopulation of thymus cells like the hydrocortisone-resistant population, which numerically is unlikely (Blomgren & Andersson 1969), and is the fact that the yield from the columns is about 50% significant, or as a result of physical factors? Zucker & Helfman (1976) have found at least four size subpopulations in the thymus, each with different properties of drug and radiation sensitivity. Ruuskanen (1975) has classified various thymus subpopulations on their alkaline phosphatase (AP) activity. The development from AP positive to AP negative cells appears to be a maturational stage correlated with the development of peripheral T cell characteristics. Soppi et al (1977) report that AP presentation may be linked to homing properties. It had been suggested that the subpopulation might be at a different "stage" of the cell cycle with respect to the whole population. Since the whole population would

exhibit cells at all stages of the cell cycle, as the rodent thymus produces about 5×10^4 cells/hour/mg of organ (Joel et al 1977), this concept suggests selection for a particular stage and there is the possibility that cells at certain stages of the cell cycle might be more mutually adhesive (C. W. Evans, personal communication). With this in mind, the experiment reported on table 9 was carried out - the filtration of thymocytes labelled with tritiated thymidine. The results of this experiment do not suggest that the column is selecting on the basis of cell cycle stage, and, although not totally conclusive, argue against the idea. The inconstancy of the values for radioactive decay per 10^7 cells are rather dubious - whether they are significantly variable, or due to counting errors when estimating the number of cells, is not known. It would have been interesting to find out what localisation patterns and adhesivenesses the other subpopulations previously mentioned displayed but this was not done.

It is still not clear what differences, other than adhesiveness and localisation patterns, the filtered cells display. However, the tentative conclusion to these experiments, as suggested by Evans & Davies (1977), was that the mutual adhesiveness of cells might be an important feature governing their localisation patterns in vivo. Reductions in mutual adhesiveness might explain how a cell can leave one organ and localise in another. Since the rate of production of cells within the thymus is so high, most cells must either die in situ or emigrate and die, the rate of production being too high to suggest that all thymocytes turn into peripheral T cells. At least some thymic migrants die in the bone marrow (Joel et al 1977) and a transient reduction in adhesiveness could

explain their release from the thymus. From a developmental point of view, it is interesting to speculate on the reason for this vast over-production by the thymus. There are at least several criticisms to this conclusion and to the experiment as a whole. Firstly, the problem of the adhesiveness of the cells in vivo as already mentioned. Secondly, is this a valid indicator system since thymocytes are not normally found in circulation and would be in a totally "alien environment"? Thirdly, is it fair to correlate the mutual adhesiveness of cells with their adhesiveness to other cell types? The problem of the indicator system will be discussed further with reference to the experiments on nylon wool produced T cells. The correlation between mutual adhesiveness and the adhesiveness to other cell types is arguable; it has already been suggested that nylon wool adhesion and mutual adhesiveness can be correlated but further work is necessary to support the hypothesis in question. The work poses great problems as it is difficult to devise an assay system which can be used to measure both parameters in the same way although intraexperiment comparisons are probably as permissable as comparisons between two different assay systems. Either way, the particular experiments have not been performed.

Having found such a result with thymocytes, it was then necessary to repeat the experiments with some other cell type which could be produced by nylon wool filtration. Thus, lymph node lymphocytes of mixed B and T cells produce a semi-pure population of T cells on nylon wool filtration (Julius et al 1973) and these can be compared to T cells prepared by another technique. These experiments have the

advantage that the cells used are found in circulation for at least some period of their life and are perhaps a rather more suitable indicator system in this respect. Initially, in the experiments reported here, B cells were removed using anti-Ig-complement lysis but this was found to be very unsatisfactory as the yields were very low. Instead, cyclophosphamide, which kills all rapidly dividing cells, was used. This is one of the problems of its use since not all T cells are long-lived and not all B cells are rapidly dividing. It was found that following cyclophosphamide treatment, the level of T cells in the lymph nodes was at least 90%. As regards adhesiveness, the CPP produced T cells are far less mutually adhesive than nylon wool produced T cells, 2.5 $\pm$ 1.16 to 10.93 $\pm$ 1.07 respectively (A. S. G. Curtis, personal communication); in the same manner as with the thymocytes, it seemed that nylon wool filtration produced more mutually adhesive cells. This result causes problems in the interpretation of the interaction modulation factor theory which has previously been mentioned and which will be discussed further.

After intravenous injection of cells in order to follow localisation patterns (see tables 10, 11, 12; figure 2), both cell types exhibited time dependent differences. In a similar study by Wiig (1976) it is stated that "T cells prepared from cyclophosphamide injected animals (spleen lymphocytes) or by nylon wool filtration of lymph node cells showed similar distribution patterns". This was not found in this study; on the whole, more of the less mutually adhesive cells remained in the blood for a period up to and including 24 hours, a result which contradicts the thymocyte data. Also, the spleen localisation of CPP

produced T cells is generally lower than the nylon wool column T cell localisation, a fact which is also evident from Wiig's data but is not acknowledged. Similarly, in his paper, Wiig (1976) states that "with the exception of a decrease in the amount of labelling found in the lungs, no distinct difference in distribution patterns was found at 24 hours after cell transfer as compared to 4 hours.". In view of the overwhelming amount of data to the contrary, presented in the results section of this thesis, this suggestion cannot be conceded.

Thus, although this data contradicts the thymocyte data and the hypothesis that the more adhesive cells remain in the blood since their adhesion to other cells is low, it is possible that the use of cyclophosphamide has clouded the results. It might have been better, in retrospect, to have produced T cells using cyclophosphamide and filter that population to give nylon wool filtered T cells but this might introduce further problems of physical trapping. So, although the mutual adhesiveness of a cell might be a guide to its localisation and recirculation properties in vivo, the concept does not receive total support in this case. It would be interesting to measure the adhesiveness of thoracic duct lymphocytes which recirculate but, although tried, it was found to be too difficult to obtain such cells. At this point in time, it is not prudent to pursue the hypothesis any further but it would seem that to explain recirculation in terms of the adhesiveness of the cell is rather too simple. As a cautionary note, it is pointed out that the use of nylon wool columns to produce pure T cell populations is not altogether valid since the column probably selects for an adhesive subpopulation.

7.4 The effects of large numbers of unlabelled cells on lymphocyte localisation

Bell & Shand (1975) found that in an adoptive transfer system, infused thoracic duct lymphocytes recirculated poorly in comparison to recirculation in a normal host. However, if a large number of unlabelled thoracic duct lymphocytes was introduced into an irradiated host prior to the labelled population, then the recirculation rate was found to be the same as that for a normal recipient. There are two (or possibly more) explanations for this result:

1. The principle of cellular space. In the irradiated host, the resident lymphocytes within lymphoid tissues will have been destroyed leaving organs such as the spleen relatively "empty". It has been suggested that cells injected into these irradiated hosts, no matter of what type, must first "fill" the available tissue space produced by the irradiation treatment before normal recirculation can occur, although this concept was not supported by experiments creating "cellular space" by chronic thoracic duct drainage. However, it is possible that thoracic duct drainage does not affect the permanently sessile population which would be destroyed by irradiation (Bell & Shand 1975).

2. The importance of the static lymphoid cell populations. In the absence of a resident population within the lymphoid organs, interactions which might normally have taken place, would not occur and these interactions could control the antibody response and/or recirculation. To investigate this hypothesis, the effect of large numbers of unlabelled cells of a single lineage on the localisation patterns of labelled marker cells was examined.

Large numbers of unlabelled thymocytes increase the recovery of labelled lymph node lymphocytes (LNL) in the spleen, not at the expense of localisation in any of the other organs sampled but rather by sequestration of cells from some other site as is reflected in the increased total recovery (see table 14). The source of the extra cells in the spleen is a mystery, especially as the recovery in the liver is also increased. The major organs not sampled in this study are the brain, heart, stomach, large intestine, muscle and skin but it seems unlikely that any of the excess cells could be explained by a deficient localisation in these organs. Even the bone marrow, which could be a potential source in terms of numbers, can be discounted. The autoradiographical data on table 16 suggests that treatment with thymocytes causes a transient hold-up of cells in the spleen particularly in the marginal zone. Direct comparisons of proportions of total cells counted in particular areas must be made with care in this case since the recovery of labelled cells in the spleens of treated animals is always higher than with the controls. The delineation of normal mouse white pulp into peripheral and T dependent areas is not only difficult but also rather subjective. Although the results shown on table 16 may not be quantitatively similar to other published data, direct comparisons can be made between data within the table as the subjectivity is due to one person.

What possible mechanisms are likely to explain this increased spleen localisation? Once released from the spleen, the extra cells within this organ must presumably be distributed throughout the sampled organs or in one or other of the non-sampled organs as, by 24 hours, the

spleen levels and the total recovery of the treated cells are not significantly different from those of the control situation. There are two possible explanations for this increased level in the spleen:

1. The excess thymocytes block the spleen traffic system so that the labelled cells cannot migrate through at the normal speed, i.e. the effect is purely physical.

2. The thymocytes alter the interaction between the labelled cells and other cells in the spleen, either directly or by the release of some diffusable substance.

The first possibility is the most likely that immediately springs to mind but there are several features that do not support it. Firstly, the injection of large numbers of unlabelled lymph node cells has no effect on the spleen recovery of labelled LNL (table 14). At 4 hours, not dissimilar numbers of thymocytes and LNL can be found in the spleen (tables 7, 14) although admittedly in different compartments (Goldschneider & McGregor 1968), and this argues against the concept that the unlabelled cells block the migratory pathway so the apparent mobility of the labelled cells is impeded. Secondly, data on table 13 shows that between 3×10^6 and 3.5×10^7 the cell number of injected labelled thymocytes or LNL has no effect on their localisation patterns, i.e. a large dose of injected cells (of the same order as used in the experiments reported on table 14) does not appear to cause non-specific trapping in the spleen. Thirdly, the different effects of excess thymocytes and B cells (table 17; to be discussed later) on the localisation patterns of other labelled lymphocyte species is not consistent with a trapping theory.

Unlabelled thymocytes do not increase the spleen localisation of labelled spleen B lymphocytes (table 20), indeed, there is a tendency that the localisation is reduced. The main effect seen on lymph node localisation which, although low in the controls, is reduced further by thymocyte treatment. This may be as a result of a failure of cells to enter the node or a speeding up of passage through the node with normal entry rates. The decreased blood levels and increased liver levels are consistent with the concept that the decreased lymph node entry is due to a paucity of cells in the blood caused by liver sequestration as occurs with neuraminidase treatment of lymphocytes (Ford et al 1976; Freitas & de Sousa 1976a) which causes less lymphocytes being available to the nodes rather than impaired recirculation through the nodes. Unlabelled thymocytes have very different effects on the localisation patterns of spleen lymphocytes (not fractionated into B and T cells). There is perhaps an increased lymph node localisation, although not significant, and a definite decrease in liver localisation which is also reflected in a decreased total recovery (table 19). This is very different to the effects on separated spleen B cells and indicates that the thymocytes are not acting on one population alone but rather altering the interactions between both B and T cells and static lymphoid populations. Unlabelled B lymphocytes, even in small amounts, have very different effects on the localisation patterns of LNL (tables 17, 18). At 4 hours, labelled B lymphocytes are found in significant amounts in the spleen (table 31), but treatment of labelled LNL with unlabelled B lymphocytes reduces the localisation in the spleen (table 17). Strangely, this result was not repeatable and the data on table 18 is quite different.

The extraordinary result is the increase in LNL localisation in the lymph nodes following treatment with B lymphocytes which was found to a greater or lesser extent in both experimental series. This result appears to be a unique case in the light of recent literature surveys (see table 3.6 in de Sousa 1976). In all other experiments on lymphocyte traffic, lymph node localisation is either reduced or remains unaltered. Also in the experiments reported on table 17, there is a significant increase in the localisation of labelled LNL in the bone marrow. Considering the total bone marrow recovery, this represents a recovery of nearly 30% as opposed to 15% in the controls. At the same time, there is a decrease in the recovery of labelled cells in the blood, but this alone cannot account for the large bone marrow difference. The effect of localisation in the bone marrow is often underestimated. In the second experimental series with unlabelled B lymphocytes (table 18), there is no increase in the bone marrow or decrease in the blood but rather an increase in the liver and total recovery. These differences between experiments are very hard to explain. That the results with treatment with unlabelled cells are not always identical is evident comparing the data on tables 14 and 15. Using ^{51}Cr as a label, the effect of unlabelled thymocytes on labelled LNL is not significantly different from the controls at 24 hours, but using $[^{3}H]$ uridine, the difference is still apparent at 24 hours and is the same as at 4 hours. This could be explained if $[^{3}H]$ uridine labels cells to different extents, as in the rat (Howard 1972) but in the mouse there is no evidence for this.

It seems that the introduction of large numbers of unlabelled cells can

alter the localisation patterns of injected labelled cells and the alteration depends on both the labelled and unlabelled species. This alone argues against a physical trapping explanation; it is suggested that the unlabelled cells alter the interactions between the labelled and other cell types causing disruptions in localisation (and perhaps recirculation) patterns. For reasons which will be discussed shortly, it is further suggested that these alterations in interaction are brought about by the action of soluble substances released by lymphocytes.

7.5 Thymus products and thymocyte interaction modulation factor (IMF)

The reports of different effector substances that are isolated from lymphoid organs and lymphocytes increases in number each week. There are factors released by non-lymphoid cells which affect lymphocyte blastogenesis (Adler et al 1978); substances released by lymphocytes which affect lymphocyte blastogenesis (Kasakura 1977); some which affect immunologic regulation (Waksman & Namba 1976) but by far the best known are the factors released from the thymus, or thymus hormones as they have been named (see Friedman 1975).

Osoba & Miller (1963) found that, following neonatal thymectomy, there were marked effects on the immunological maturation of the animal which could be repaired by thymus grafts but not thymus lymphocytes alone. It seemed that the substance necessary might be derived from the epithelio-reticular network comprising the skeleton of the thymus. Later, the purification of a thymus hormone, thymosin, was announced by A. Goldstein & co-workers (1972), a substance of molecular weight around 12,200 and of 108 amino acid residues

(Hooper et al 1975) which has been shown to be produced by the thymus epithelial cells (Mandi & Glant 1973). Thymosin was thought to consist of several protein subunits, some of molecular weights as low as 1,000 daltons. J.-F. Bach & co-workers, over the past few years, have isolated a circulating thymus product, again from thymus epithelial cells, which has a molecular weight around 900-1,000 daltons and which disappears on ageing (Bach & Dardenne 1973, Bach et al 1972, 1975, 1977). Other thymus products that have been isolated include thymin, renamed thymopoietin (G. Goldstein 1968, 1974, 1975). This substance is also produced by thymus epithelial cells and consists of two polypeptide types with molecular weights around 7,000 daltons. The effect of thymopoietin is to alter neuromuscular transmission which connects it closely with the neuro-thymic disease, myasthenia gravis. A third polypeptide type, isolated during the preparation of thymopoietin (G. Goldstein 1975), has a molecular weight of 4,000-12,000 daltons but does not affect neuromuscular transmission. It is widely distributed in all tissues of many species and has been called "ubiquitous immunopoietic polypeptide (UBIP)". Another small (3,000 daltons, 30 amino acid residues) substance (THF - thymus humoral factor) has been isolated (Trainin & Small 1970, Kook & Trainin 1974, Yakir et al 1978) which affects T cell immunocompetence by altering intracellular levels of cyclic adenosine monophosphate. The point of mentioning these thymus products is to pave the way for discussion on a thymus product which has been isolated by Curtis & de Sousa (1973, 1975).

It has been found that supernatants of cultures of sponge cells from one

particular strain type can decrease the adhesiveness of cells from another strain type (Curtis & van de Vyver 1971). In the same way, it was also found that supernatants of T cells could decrease the adhesiveness of B cells and vice versa in human, rat and mouse systems (Curtis & de Sousa 1973, 1975). These substances have been called "interaction modulation factors" (IMFs). IMFs from T cells and thymocytes appear to be functionally identical and both differ from B cell IMF. Recently, and in this thesis, most of the work has involved characterising thymocyte IMFs in a syngeneic system. Thymocyte IMF was originally thought to be a protein (Curtis & de Sousa 1975) but it now seems that it is probably a glycoprotein (Curtis et al 1978) with the active groups being proteinaceous. The original molecular weight determination of 9,000 daltons was made with respect to standard globular proteins on a polyacrylamide gel-filtration column but in the light of the new evidence that the IMF is not totally protein, this value is probably incorrect.

Is thymocyte IMF unique among the thymus products or is it allied to any of the other substances previously mentioned? Unfortunately, all the assay systems are different and no comparisons have been made but there are two major differences which suggest that thymocyte IMF is a novel isolation:

1. The method of production. Thymosin (A. L. Goldstein), thymus humoral factor (Trainin) and thymopoietin (G. Goldstein) are all isolated after homogenisation of whole calf thymus followed by various extraction processes. Thymocyte IMF is made by culturing thymocytes (thymus lymphocytes) of high viability in

serum-free culture for a short time. It is now certain that thymosin, THF and thymopoietin are derived from thymic epithelial cells (Mandi & Glant 1973, Goldstein 1975, Blankwater et al 1978) and an antithymic reticuloepithelial cell serum has been found to reduce the levels of a circulating thymic factor and to increase the sensitivity to azathioprine of spleen spontaneous rosette-forming cells (Garaci et al 1976), a test which has been used to assay thymosin and other thymic epithelial cell products (Bach et al 1971). A supernatant from thymocytes alone was inactive compared to thymosin in an in vitro antibody production system (Blankwater et al 1978). Since the contamination by other cell types in thymocyte suspensions used in the production of thymocyte IMF is very low, it would seem that from the production aspect alone, thymocyte IMF is different from other thymus products.

2. The biochemistry of various thymus products. As mentioned, most of the thymus products isolated have molecular weights between 1,000 and 12,000 daltons, although some are obviously subunits. Dardenne et al (1974) and Bach et al (1977) have isolated a nonapeptide serum thymic factor from epithelial cells of molecular weight 900 daltons, which has the same functional effects as whole thymosin on the sensitivity of spleen B cell rosettes to azathioprine. No other thymus cell products have been stated to be glycoproteins other than thymocyte IMF which stains positively with periodic acid-Schiff, a glycoprotein stain, after polyacrylamide gel-electrophoresis (A. S. G. Curtis,

unpublished observation) whereas there is no staining with Coomassie Brilliant Blue (personal unpublished observation), a stain which is specific for proteins rather than glycoproteins.

There are some other thymus products reported which have similarities to thymocyte IMF. A carbohydrate extract of 12,500 daltons produced during the thymosin purification process has also been shown to slightly increase the responsiveness of thymocytes to T cell mitogens, while maintaining some of the characteristics of thymosin itself (Burke & Ambrus 1976). Ernstrom & Norlind (1977) have isolated a thymus factor (probably in several components of molecular weight less than 10,000 daltons) that stimulates DNA synthesis in cultured lymphocytes. Conversely, Kiger et al (1977) have purified a substance which inhibits the DNA synthesis in short term cultures of mouse thymocytes (molecular weight 10,000-50,000 daltons). Kruisbeek et al (1977, 1978) have found that a thymic epithelial cell culture supernatant increases the T cell mitogen responsiveness of cortisone-sensitive thymocytes. Malyzhev (1977) has reported a "low molecular humoral factor of the thymus" which decreases the responsiveness of thymocytes to a T cell mitogen. In common with some of these observations, thymocyte IMF has been found to reduce the responsiveness of lymphocytes to T cell mitogens but only when the IMF was in a semi-pure state. Fully purified IMF (with regard to maximum de-adhesion) had no effect on the responsiveness of lymphocytes to mitogens, indicating that this component was probably a contaminant of early preparations (W. Haston, unpublished observations). Also, de Somer

et al (1963) have found that a calf thymus culture extract can restore the peripheral lymphocyte count in neonatally thymectomised mice. Thymocyte IMF has also been found to induce a leucocytosis in mice, (see table 23).

The present position as regards thymus products is then very confused. It is obvious that many of the substances are one and the same but due to the multiplicity of assay systems, they have been classified as being novel substances. The major division is between products of thymic epithelial cells and those of thymic lymphocytes - the majority of thymus products so far isolated have been from whole thymus extracts but most have been proven to be epithelial in origin. It would seem that thymocyte IMF is lymphocytic in origin although the possibility that some epithelial cells might contaminate the cultures cannot be ruled out. If a contamination rate in cultures is estimated as 0.1%, then approximately 5×10^5 epithelial cells might be found in each culture of 5×10^8 cells which could explain the apparently small quantities of IMF released. However, this possibility seems unlikely as does the suggestion that IMFs are a product of dead cells. Whether these substances are constantly produced or cleaved from the cell membrane and not replaced is not clear, although there is some evidence that in cycloheximide treated thymocyte cultures, little or no IMF is produced (unpublished observation).

A final and most important question is whether IMFs exist naturally in vivo or whether they are in vitro artefacts. The evidence in support of the former is somewhat circumstantial - B and T cell IMFs are found in normal serum (or at least something which mimics their effect),

and the B cell IMF level is very much elevated (with respect to the T cell IMF) in the serum of the nude mouse, which lacks mature T cells (Curtis & de Sousa 1975). There is also some evidence that T cell IMFs are elevated in certain human disease states, namely mycosis fungoides and chronic lymphatic leukaemia (Curtis, unpublished observations). Thymocyte IMF is also either produced or bound by other tissues in the body (Curtis 1978a), not unlike G. Goldstein's UBIP. The relevance of the latter finding is hard to imagine and the serum interpretations must be made with care in the light of the observation that serum reduces the adhesiveness of cells anyway (Curtis & Greaves 1965).

7.6 The effects of thymocyte IMF in vivo

In the experiments conducted in this study, purified mouse thymocyte IMF has been used in various different syngeneic assay systems. It was decided to remain within the confines of a syngeneic system since any IMF used would not be recognised as a foreign antigen. Further, it has been found that thymocyte IMFs from strains of mice that are not matched at the H-2D locus affect the adhesiveness of thymocytes as well as B cells from the mismatched strain (Curtis 1978a). The IMFs used have varied greatly in activity, probably a reflection on the purity of the preparations (see table 21). The activity of the IMF is a measure of its capacity to reduce the adhesiveness of B lymphocytes and does not appear to be directly correlated with the protein content of the preparation. In view of the recent finding that thymocyte IMF is probably a glycoprotein, the classification of the sample by protein content may not be of much use. T IMF 127 appeared to be about ten

times as active as most other preparations and this can probably be explained by an improvement in the purification techniques. Similar, and even greater activities have been produced in subsequent batches.

Thymocyte IMF 127, because of its high de-adhesion activity, was also tested for toxicity on LNL. This perhaps was not the best cell type to have chosen since LNL are known to lose radioactive chromate label rapidly in culture (Sanderson 1964). Despite this, there is little indication that the IMF is toxic particularly over the course of two hours. It is, therefore, felt that the in vitro incubations with IMF of 15-30 minutes prior to most in vivo experiments does not compromise the subsequent viability of cells.

The effect of thymocyte IMF in vivo has already been briefly mentioned. When given intravenously in an isotonic solution, thymocyte IMF causes a rapid leucocytosis in the blood which is evident as early as 15 minutes after injection (data not shown). At 2 hours, the total leucocyte level in the blood is significantly increased (table 23) and it seems from the differential counts that no particular leucocyte type is specifically enriched. At the same time, there is an increase in the overall ratio of Ig positive cells in the blood and a decrease in Ig positive cells in the spleen. This could be explained in two ways:

1. The specific trapping of blood-borne non-Ig positive cells in the spleen.
2. The release of Ig positive cells from the spleen into the blood.

Histological examination of the spleen after IMF treatment showed the red pulp to be relatively depleted of lymphocytes and PML, whilst the

white pulp appeared normal (there was some indication that peripheral areas of the white pulp might be depleted but not to any significant degree). This effect was not seen in the spleens of saline injected controls nor was it produced by the rapid exsanguination process of obtaining blood. In view of these observations, it is suggested that the second hypothesis is the more likely and the appearance of a non-specific blood leucocytosis is consistent with the release of cells from the splenic red pulp. A problem with determining the lineage of spleen lymphocytes is the presence of so-called "null" cells. These cells lack detectable amounts of surface Ig and Thy 1 antigen and thus cannot be classified as B or T cells. Typically, a mouse spleen might be expected to contain 38% B cells, 35% T cells and 27% "null" cells (Hudson & Hay 1976). If only "null" cells were released from the spleen by IMF treatment, then the apparent ratio of Ig positive to Ig negative lymphocytes would fall (in these experiments, the percentage of Thy 1 positive cells was not measured). This theory is not consistent with the observed rise in the Ig positive lymphocyte level, although it is not known what effects are occuring to the T cell population. Peripheral blood itself only contains around 6% "null" cells so sequestration of this particular cell type cannot entirely explain the large apparent decrease in Ig positive lymphocytes observed in the spleen.

Twenty four hours after IMF injection, the blood leucocytosis is no longer present but the differential counts of PML to lymphocytes shows a strange unexplained inversion. There is a possibility that the results for the differential leucocyte counts are artefactually incorrect. The gelatin sedimentation method for making white blood cell smears is

probably an unreliable technique, in that leucocytes can be trapped in the red blood cell rouleaux. However, the percentages of Ig positive cells in the blood and spleen still remain altered in the absence of any detectable leucocytosis. This point is also difficult to reconcile. In all probability, any PML released would be removed from blood circulation within 24 hours, their lifespan being relatively short. Thus, any lymphocytes released might either still remain in circulation or lodge in some other organ. The percentage of Ig positive cells counted in the blood suggests that the released lymphocytes have remained in circulation, while the blood leucocyte count suggests that released cells have been removed from circulation. These two hypotheses are incompatible unless the IMF is having an additional effect on circulating lymphocytes. For instance, thymocyte IMF, making B cells less adhesive (to themselves and possibly to other cell types), may cause them to remain in circulation rather than lodge in lymphoid and non-lymphoid organs. Similarly, the relative depletion of B lymphocytes in the spleen may allow T lymphocytes to become more adhesive and hence remain in that organ. In addition, thymocyte IMF is found to have no effect on both the cellularity and the Ig positive to Ig negative lymphocyte ratio of peripheral lymph nodes (data not shown). What effect the IMF is having is certainly not clear and any suggestions are pure conjecture in the lack of any further experimental evidence. Accurate differential leucocyte counts and longer time courses of the experiments might provide additional useful data; also, the effects of a B lymphocyte IMF, when purified, will be interesting.

Having found that injected thymocyte IMF has a profound effect on leuco-

cyte flux within the spleen, it is of further interest to see the effect of injected IMF on the localisation patterns of injected labelled lymphocytes. Tables 24-28 show the effects of various different thymocyte IMF preparations, used in different quantities, on the localisation patterns of labelled LNL. IMF 122 (table 24) after one hour depresses the localisation of LNL in the peripheral lymph nodes, but curiously not in the mesenteric nodes. At the same time, there are tendencies for the spleen and liver localisations to increase, although these are not significant due to the small sample numbers. Again, as with the thymocyte treated LNL localisation data, there is also an apparent increase in total recovery. By 4 hours after injection, the increases in the liver and spleen are significant and the decrease in the peripheral nodes still apparent. The localisation in the mesenteric nodes is again unaffected, a point which is very hard to explain. The input population of LNL consisted of mixed peripheral and mesenteric node lymphocytes and it would have been interesting to compare the effect of IMF on separate peripheral and mesenteric lymphocyte populations. In the mouse (and in the rat and sheep), activated T cells (lymphoblasts) from the mesenteric nodes localise preferentially in the lamina propria of the small intestine, while peripheral T lymphoblasts localise in the spleen - a difference which was not found with normal unactivated mesenteric and peripheral T lymphocytes (de Freitas et al 1977). Perhaps, in this case, the divergent pattern of localisation is due to B lymphocytes but data on table 29, although using spleen B lymphocytes as target cells, does not substantiate this hypothesis. Also, at 4 hours, there is a significant drop in the bone marrow localisation and notably no alteration in the number of cells remaining in the blood. Again,

as suggested before, the deficient lymph node localisation might be explained by a decreased availability of cells due to the increased localisation in the spleen and liver. The lack of any difference in the blood and mesenteric nodes makes this possibility unlikely. Note also the increased total recovery, the source of which is again unknown. Twenty four hours after IMF 122 and labelled LNL injection, no significant differences in localisation patterns are detectable.

In this experiment, 7,000 active units of thymocyte IMF were injected compared to 20,000 active units in the experiments reported on table 23 on the blood leucocyte effects of IMF. It is important to consider the altered localisation patterns that are observed, in the light of the effect of injected IMF. Free injected IMF causes a release of cells from the spleen, causing "free space" to be available to incoming cells. It is possible then, that injected labelled LNL might be caused to recolonise areas of the spleen in the presence of IMF, although the IMF itself might clear these cells themselves. Autoradiographical analysis of injected LNL localisation in the spleen after IMF treatment (table 27, but note that the IMF used is a different batch and in a different quantity, from the point of view of activity) indicates that cells do not remain in the red pulp but may be held up in the marginal zone and peripheral areas of the white pulp. This does not support the suggestion that the increased number of injected LNL localising in the spleen colonise the area depleted by the IMF treatment, although the IMF may itself be responsible for clearing the red pulp. As with the autoradiographs of the spleen following thymocyte treatment, the percentage of cells localising in the particular areas is not altogether the best representation of the situation since the overall number of cells in the spleen is increased.

IMF treatment would appear to cause an increase in traffic speed through the spleen. The increased localisation of LNL in the liver following injection with IMF is again hard to explain. IMF does not appear to greatly compromise the viability of LNL so it seems unlikely (although not impossible) that the extra cells are dead. B cells localise to a greater extent in the liver than T cells (e.g. tables 10, 11, 20, 29) so it is possible that the cells localising in the liver over and above the normal levels are B lymphocytes. Thymocyte IMF, affecting B lymphocytes, might alter their interaction with lymphoid and non-lymphoid cells within the liver but this point would be very hard to resolve - analysis of the small number of lymphoid cells in the liver being technically difficult.

The deficient peripheral lymph node localisation is easier to explain, although the paradoxically unaffected mesenteric node localisation is not. Thymocyte IMF causes the release of leucocytes into the blood from the spleen while the lymph nodes are unaffected, probably as the IMF does not actually enter the nodes. With a greater number of lymphocytes in the blood, the ratio of labelled injected lymphocytes to host blood lymphocytes will therefore fall after IMF treatment. In this case, the actual number of labelled cells in the blood will be unaltered but the number of labelled cells entering the node will decrease since there are a greater number of unlabelled cells entering. This is not competition but simply a case of a dilution of the number of labelled cells available. However, having put forward a plausible explanation for the decreased localisation, the same cannot be said for the mesenteric nodes, at least in this experiment, but this problem is not apparent in all the experi-

mental series and the discussion will be resumed again with respect to the effects of other IMFs. The deficient localisation in the bone marrow is a different matter, and a plausible explanation hard to find. Further investigations as to the effects of IMF on the bone marrow would be necessary but again, this finding is peculiar to the particular experimental series using IMF 122.

A similar experiment was repeated using IMF 124 on labelled LNL. Although the same weight of protein was used, the activity per unit weight was nearly four times greater. It is thought that better and more careful purification resulted in this increase in activity. The time courses used in this particular experimental series were 2, 4 and 8 hours (tables 25 and 26) but unfortunately, mice from two different sources were used for the different times with the result that some of the data is incompatible. The use of the two mouse suppliers in the one experimental series was unavoidable and was due to a shortage of mice from the normal supplier at the time of the experiments. By the time more mice were available, stocks of IMF 124 were depleted. Even still, the four hour control data for mice from the same source, on tables 24 and 25, are not completely compatible. The effect of IMF 124 on labelled LNL is somewhat different to that of IMF 122; the increase in spleen and decrease in peripheral node localisations are again apparent, but in this case there are no alterations in the liver or bone marrow recoveries. Instead, there is a decrease in the localisation in the lungs. A feasible suggestion for this is that the IMF has not only affected lymphocyte-lymphocyte adhesion but also has decreased lymphocyte adhesion to other cell types. The hypothesis further suggests that the lung difference is due to a higher mobility of B lymphocytes, leaving T

lymphocytes remaining. The explanation of the apparently deficient peripheral lymph node localisation could be the same as previously suggested, but the differences in bone marrow and liver localisation, between the two experiments, suggest that either the IMF action is very transient, or it is negatively dependent on activity, or the preparations contain different active contaminants. More extensive biochemistry has only been performed on IMF 127, so the latter suggestion cannot be proved. The paradoxical mesenteric lymph node localisation is again apparent.

Also, as shown on table 25, the effect of a thymocyte conditioned medium - i.e. an impure thymocyte IMF preparation, low in activity - on LNL localisation was measured. It can be seen that the conditioned medium has a similar, though less pronounced, effect as the IMF and a similar effect to an added population of complete thymocytes (see also table 14). Large variances in the data do not permit statistical analysis.

The effect of IMF 124 at different times on mice from another source is shown on table 26. Although used at the same activity on the same target cells, the results are totally different. At 2 hours after injection of IMF and LNL, there is a significant increase in liver and decrease in both peripheral and mesenteric lymph node localisations. These findings are also apparent at 8 hours to a greater and lesser extent. Why this difference should occur is difficult to understand. The increased liver localisation in this instance does suggest that the cells are in fact dead or dying and may be the cause of the deficient lymph node localisation. These strange results are a good example of the problems that have been encountered using IMFs <u>in vivo.</u> As previously mentioned, the effect

of IMF 124 on the intrasplenic distribution of labelled LNL was also measured (table 27, cf table 25). The results are qualitatively similar to those of LNL localisation with thymocyte treatment (table 16) possibly indicating a faster passage of cells through the spleen or alternatively a trapping effect in the white pulp.

Thymocyte IMF 126 when injected with labelled LNL again has slightly different effects to the two other IMFs previously tested, even though used in similar assay and protein quantities to IMF 124. Using IMF 126, there is a significant increase in liver localisation and decrease in lymph node and small intestine localisations after 4 hours. No longer is the spleen level elevated and notably, the total percentage recovery remains the same. The blood level is slightly depressed, although not significantly. Perhaps, in this case, the explanation for the deficient lymph node localisation, this time in both peripheral and mesenteric nodes, is that increased liver localisation has resulted in a reduced number of labelled cells in the blood (although not significant) and this, coupled with the increased number of unlabelled lymphocytes in the blood produced by the IMF itself (all the IMFs tested did produce a blood leucocytosis), causes the reduction in the lymph node localisation. The reduction in the small intestine, which is apparent to a greater or lesser extent with all the IMFs (see table 30) is less easy to explain. It is not known whether this decrease is due to deficient localisation in Peyer's patches or in the lamina propria of the intestine itself, and again it may be the result of the increased localisation in the liver rather than a specific effect.

The experiments so far have been concerned with the effects of IMFs injected <u>with</u> labelled cells and thus take into account the overall effect

of the IMF in vivo. The binding of thymocyte IMF to B cells is either weak or transient as several washes in medium is enough to restore the adhesiveness of the target cells (A. S. G. Curtis, personal communication). Thus, cells treated in vitro with IMF were not washed prior to injection, unlike the enzyme experiments previously reported in which the cells were well washed before use. However, the remaining data on table 28 shows the effect of one wash in excess medium on the localisation of labelled LNL. This will effectively dilute any IMF in the injection medium to a minimal and probably sub-active level. Bearing in mind the fact that the binding may be weak and the effect of the IMF in vivo, the results are surprising in that they are both quantatively and qualitatively similar to the results with IMF injected with the cells. Unfortunately, the variations in spleen recoveries are so great (for some unknown reason) that any differences may not be apparent. These results indicate that the IMF localisation alterations on labelled LNL are probably due to the labelled population itself as well as gross in vivo effects and that the effects of the in vitro incubation are possibly the more important.

The effects of injected IMF on labelled LNL localisation for the three IMFs tested are qualitatively compared on table 30. Few features are common to all three preparations except a tendency for decreased localisation in the small intestine. The results indicate that some component in the IMF preparations must be variable, either in content or activity and thus, it is probably true that, despite the techniques used at present, the preparations are not yet really pure enough for accurate in vivo use.

IMF 122 was also tested for its effect on the localisation patterns of labelled spleen B lymphocytes. The data on table 29 shows that IMF treatment again causes an increased localisation in the liver at both 2 and 4 hours, which is also reflected in large increases in the total recovery. Similarly, there are significant decreases in lymph node localisation at 4 hours, but no differences in spleen, bone marrow, blood and small intestine localisation. This data can be compared with that on table 20 on the effects of a large number of thymocytes on labelled SBL* localisation except that in the latter, there is no increase in the total recovery. The fact that excess thymocytes, as well as IMF, can increase the liver localisation makes it unlikely that the increased numbers of cells are dead but more likely that some form of cell interaction has been altered.

* SBL = spleen B lymphocyte

7.7 Concluding remarks and some speculations about IMF action

So, in the last analysis, we are left with several important questions:

1. Is the present system, used by others and in this study, to examine recirculation a valid physiological example of the true mechanism in vivo?

2. What have we learned from these experiments reported in this thesis?

3. Can interactions between lymphoid cells really provide a feasible mechanism for the overall patterns of lymphocyte circulation?

There have been three basic experimental approaches in the study of recirculation:

(a) By measuring the kinetics of blood to lymph recirculation of cells as initiated by the work of Gowans.

(b) The examination of the gross localisation patterns in various organs of treated and untreated lymphocytes, as in the enzyme treatment experiments.

(c) Using isolated perfused lymphoid tissue like the spleen and lymph nodes to measure the kinetics of cell passage through those particular organs.

None of these experimental approaches can be described as truly physiological and each has only dissected away a small part of the whole in the hope of providing a clue to the overall mechanisms involved in recirculation. True, we do now have more insight into some of the selection mechanisms which act in the lymph nodes - we know how important the spleen is in maintaining lymphocyte flux within the recirculating pool but in our attempts to examine supposedly discrete areas of the lymphoid system by using in vivo techniques, we have tended to overlook the effects of the lymphoid system taken as a whole. Recirculation is a dynamic and finely balanced process involving most of the major organs in the body, not just an endless flow of cells from blood to lymph and back into the blood. In the recirculation experiments, only a small number of labelled cells are examined by their appearance in the lymph - but what happens to them after they leave the lymph? This question is often neglected. A major criticism of the overall localisation studies, as in the work of Freitas & de Sousa and the work in this study presented here, is that on every occasion we

introduce large numbers of cells into circulation which are normally sedentary. The vast majority of thymocytes are never destined to even enter the bloodstream - most lymph node and splenic lymphocytes spend a good deal of their lives as sedentary cells and we hope that they can tell us about the mechanisms of recirculation. What we can learn, and what appears very clearly in this work, is that rather than showing us how recirculation through the nodes occurs, they show how important cell interactions within the whole body are in controlling the movements of cells. To comprehend the vastly complex mechanism of cell movements, we must take into account all the interactions which can take place in vivo, not only with other cell types but also with substances in the circulatory systems. When discussing the majority of experiments designed to elucidate the mechanisms of recirculation, we must bear in mind that few are physiological and few provide us with data that is easy to interpret.

What questions has the work in this thesis answered and what contribution to the understanding of lymphocyte circulation has been made? The work that has been done is broadly in three sections:

1. The influence of like-like cell adhesiveness on in vivo lymphocyte positioning.

2. The effect of large numbers of unlabelled lymphocytes of a single lineage on the positioning of other lymphoid cells.

3. The effect of purified thymus cell products on the positioning of lymphocytes.

The concept (see section 7.3) that the measurement in vitro of the adhesiveness of a particular cell can be correlated directly to its in vivo positioning patterns now seems rather naive. In the case of the thymocyte subpopulation studies, it is very likely that the nylon wool column selects for a particular subpopulation with different positioning characteristics and that there are many other differences in the cells than just their mutual adhesivenesses. This is substantiated by the finding that CPP produced T cells and nylon wool column produced T cells behave in ways which would not be predicted from an extrapolation of the thymocyte subpopulation data. In this case, it would have been better to have compared the localisations of CPP produced T cells with CPP nylon wool column filtered T cells as was mentioned earlier, although the minimal cell yields from CPP treated animals, coupled with a further feduction in yield from the nylon wool columns would have made this experiment difficult. A change in the adhesiveness of a cell is unlikely to occur as a mutually exclusive event but rather coupled with some other change, like an alteration in the cell cycle state. It is thus impossible to compare directly the positioning in vivo of identical cells which only differ in their mutual adhesiveness. This would hold true not only in the case of lymphoid cells but also with any other cell type studied.

Large numbers of lymphocytes of a single lineage have been shown to alter the positioning of different types of lymphoid cell in a way which cannot be attributed to any physical effect of cell trapping. The primary conclusion from these results is that the interactions between lymphoid cell types, both sedentary and circulating, are of major importance in

controlling the movements of lymphocytes. That this is true is also indicated by comparing the localisation patterns of cells of different lineages produced in vitro from lymph nodes. The localisations of lymph node lymphocytes (a mixed population of approximately 65% T cells and 35% B cells) cannot be predicted by simple proportional comparisons using the localisations of complete single populations of T and B cells (tables 11, 24, 31). It has been found that lymphocytes release substances which can alter the interactions between other lymphoid cell types (Curtis & de Sousa 1973, 1975) but these observations were confined to in vitro measurements. The third section of this work examined the possibility that these substances (IMFs) could explain the differences in localisation patterns shown using the large doses of single lineage cells.

As far as they have been studied, it appears that the actions of IMFs can explain alterations in localisation patterns and certainly, in the case of thymocyte IMF, the effects are not dissimilar from those using large numbers of whole thymocytes. Whether the same can be said for B cell IMF remains to be seen.

A process during recirculation, for which little explanation has yet been put forward, is stage 3 - the sorting-out of recirculating cells within the node into T and B dependent areas. Take, for example, the possible routing of lymphocytes having just crossed the HEV barrier into the node. The cells enter the node and remain together for a while in a T dependent zone, beginning segregation 1-6 hours after entering the node (Nieuwenhuis & Ford 1976). B cells can be stimulated to move (chemokinesis) by thymocyte (and probably T cell) IMF

(Curtis et al 1978) and thus would be able to move away from the T dependent area where they previously resided. The further action of the T IMF, reducing the adhesiveness of B lymphocytes, would prevent moving B cells aggregating to form B cell foci within T dependent areas. T IMF may also reduce the interactions between B and T lymphocytes (de Sousa & Haston 1976) which would further assist segregation. Thus, a B lymphocyte would remain motile until it reached a B dependent zone. Here, one would have to assume that the action of the T IMF might be decreased, perhaps by a cancellation effect caused with an interaction with B cell IMF, which would be produced in large amounts in B dependent areas. The B cell would then be in the right environment to cease moving and form a stable adhesion with cells of the same type. Interaction with antigen might alter the timing and pattern of B cell movement through the node. A similar mechanism might be postulated for the movement of T cells within a node, except that they arrive in a T dependent area on entering the node and can form an immediate adhesion with cells of a like type. The release of recirculating cells into efferent lymph is a difficult process for which to suggest any mechanism. Obviously, the timing of release or route of exit of the cells is such that B cells take much longer than T cells, which would explain the difference in recirculation times.

In the previous paragraph, a mechanism causing segregation of B and T cells within the lymph node was proposed in terms of interaction modulation factors. These substances were first postulated in a theoretical analysis by Edelstein (1971) to explain the sorting-out of cells

in a morphogenetic system and were thus named "morphogens". Curtis & van de Vyver (1971) showed the existence of such morphogens which controlled coalescence of strains of a freshwater sponge, Ephydatia fluviatilis. Morphogens have now been renamed "interaction modulation factors" by Curtis (1978b). The IMF theory has been put forward as an explanation for the specific control of cell positioning but there are several other theories that have also been suggested to explain the formation of adhesions between cells and sorting-out. None have ever been used to interpret data concerning lymphocytes, so it is worth mentioning them here to see whether any of the postulated mechanisms might be relevant.

Perhaps the most well known of the theories of sorting-out is Steinberg's differential adhesion hypothesis (DAH) which was first proposed some sixteen years ago (see Steinberg 1978 for review). The crux of the DAH, as predicted from the behaviour of immiscible liquid systems, is that the adhesiveness displayed between like cell types will determine their position in an aggregate formed from single cells. Working with chick embryonic tissues of limb bud, heart and liver, Steinberg found that limb bud sorted-out internally with respect to heart and liver and heart sorted-out internally to liver - known as "sphere-within-a-sphere" configuration. The reasons for these results were assumed to be that adhesions between limb bud cells were greater than those between like heart or liver cells and the intratypic adhesions were greater than any of the intertypic adhesions. It should be pointed out that measurements which reflect the net rate of initiation of adhesiveness among or between cells (as in the Couette viscometer, Curtis 1969)

might not necessarily reflect the actual energies of adhesion. Thus, in one system, limb bud cells may be the most adhesive but may also have the lowest aggregation rate (Steinberg 1970). The differential adhesiveness of cell types, which is necessary to explain sorting-out in this hypothesis, is brought about through the regulation in frequency of individual adhesive "sites" distributed at random over the cell's surface.

What neither the DAH nor the morphogen/IMF theories of sorting-out answer is the actual mechanism by which adhesions are formed and maintained, they merely demonstrate how specificity can arise. There are several theories on how adhesions are maintained, some are chemical, some are purely physical. It has been known for some time that there are species-specific aggregation-promoting factors produced when seawater sponges are dissociated into single cells using calcium- and magnesium-free seawater (Humphreys 1963). These substances, aggregation factors (AF), have been partially purified and have molecular weights of around 2×10^7 daltons consisting primarily of 2×10^5 dalton subunits (Cauldwell et al 1973). It has been suggested that glucuronic acid-like residues on the AF are recognised by some component of the cell surface of dispersed cells (Turner & Burger 1973). This component can be released by osmotic shock from the cells and has been called "baseplate" (Weinbaum & Burger 1973). Thus, adhesions between cells can be brought about by interactions between AF and the cell surface bound baseplate, a process which requires the presence of calcium and magnesium. Working on the freshwater sponge, Ephydatia fluviatilis, Curtis & van

de Vyver (1971) have isolated an AF of much smaller molecular weight than the seawater sponge factor, which not only enhances aggregation of like strain types but inhibits the aggregation of unlike strain cell types. Similarly, chick neural-retina tissues have been found to release a substance which contains a tissue type-specific component which enhances aggregate size (see Lilien et al 1978). It has been suggested that although some of the data is compatible with the neural-retina factor being an intercellular ligand, like the sponge AF, the requirement for metabolic activity for cell reaggregation also implies some catalytic role in stimulating the synthesis/action of adhesive components (Lilien et al 1978).

Roseman (1970) has suggested that surface glycosyltransferases are necessary mediators for adhesion and interact with oligosaccharides on other cells to form an adhesive bond. The specificity for adhesion would arise from enzyme specificities. Sufficient practical evidence for surface glycosyltransferases has yet to be provided. The specific ligand theory of Lilien et al (1978) is not incompatible with this model; they suggest that the surface ligand exists in an inactive form and following activation by an intracellular glycosyltransferase, the ligand becomes free to interact and form an adhesive bond. Feedback controls on the rate of activation and release of the ligand could modulate the stability of adhesions.

The mechanisms for adhesive bonds that have been mentioned so far require the formation of chemical bridges between cells. The problem arises that the distance between the outer surfaces of adherent cells is about 10-20nm in some instances, while in other cases, such as the

"tight" junction, the cells are at least an order of magnitude closer to each other. Can any molecular bridge system exist over a distance of 10-20nm? To explain this, the electrostatic forces of attraction/repulsion between cells needs to be considered. Curtis (1973) has expounded the DLVO (Deryagin, Landau, Verwey & Overbeek - after the originators of the work) theory to explain the electrostatic interactions between cells. All cell surfaces are negatively charged, thus, cells which are close together will be repelled. The repulsive force is dependent on the reciprocal of the ionic strength of the medium and declines exponentially with distance away from the surface. The physical adhesion of cells needs attractive forces - London-van der Waals' forces, which depend partly on the polarisation of net charges due to electron fields of the molecules of the cell surface. Resolving the various formulae of repulsion and attraction, it is found that there are particular distances where two cells will be attracted together rather than repulsed. These distances are consistent with tight junctions and the 10-20nm gap previously mentioned. Between these values, cells are repulsed but how cells are able to overcome the energy barrier to form tight junctions is a further problem. Whether these physical parameters can really be applied to a system as complex as the cell surface is difficult to evaluate but certainly electrostatic interactions must be important at some stage in adhesion.

Having discussed the possible mechanisms for adhesion and some mechanisms for specificity, can the two systems be correlated into one united concept? Steinberg (1978) considers that his DAH is compatible with the cell ligand theory of Lilien and co-workers. He

suggests that although there is probably more than one type of adhesive site, one of which may be common to many cell types, differential adhesion is still due to the number and distribution of adhesive sites rather than direct chemical differences. The morphogen/IMF theory has not been connected, so far, with any particular adhesive mechanism. Unlike aggregation factors and theoretical cell ligands, IMFs, as in the lymphocyte case, reduce the rate of aggregation and perhaps adhesiveness of single cells. In order for this theory to be correlated with the ligand hypothesis, more knowledge about the biochemistry of action of IMFs is necessary.

The discussion, in the last few pages, of the theories of cell adhesion might not seem to be directly relevant to lymphocyte circulation. However, having suggested the IMF theory as a suitable explanation for sorting-out within the lymph node, it is necessary to see whether any of the other theories can also explain the phenomenon adequately.

One problem with comparing sorting-out within the node and classical embryonic sorting-out is that the former is a transient movement, whereas the latter is more or less permanent. The breaking of the adhesion of a T cell from its neighbours in a T dependent area to subsequently enter the efferent lymph is difficult to explain in terms of any of the theories previously discussed. Can the DAH explain the movements of lymphocytes within the node? As Steinberg (1978) himself says, the DAH is a physical hypothesis concerned with the strengths of adhesion, while the IMF theory (and the practical results) are more concerned with the rate of adhesion, two separate measurements that are not necessarily connected. On the whole, T dependent

areas are usually enclosed by B dependent areas (or perhaps, rather, non-T dependent areas); in terms of the DAH, the sorting-out of T cells could be likened to the limb bud/heart/liver situation where limb bud, having greater strength of adhesion, sorts-out internally to heart and liver. Only the rate of initiation of adhesions is given in the data of Curtis & de Sousa (1975) suggesting that T and B cells are similar. However, the aggregation rates for nylon wool produced T cells and CPP produced T cells do not corroborate this (see section 7.3). The IMF theory requires chemokinetically induced movements during sorting-out but no mention is made of directed cell movements being involved in the DAH. Both the DAH and IMF theories could be used to explain emigration from the node if some step of de-adhesion is assumed - either a build-up of IMF of the opposite type or a chemical change in the adhesive mechanisms.

A process which may be important for sorting-out within lymphoid organs is chemotaxis, the guided directional movement of cells. T cells could be attracted towards T dependent areas by chemotactic agents which would have to be different from those released from B dependent areas to provide the necessary specificity. Although lymphocytes have been shown to respond to certain chemotactic agents (e.g. Ward et al 1971, 1977), there is no evidence yet in favour of specific attractants for T and B cells that can explain sorting-out. IMFs have been found to induce chemokinesis (non-directed movement) in cells of the opposite type (Curtis et al 1978) but it is not yet certain whether they can induce chemotaxis as well. Chemotactic reactions of cells within preformed organs (rather than during the formation of an organ) are not consistent with the DAH.

There is no clue to the actual mechanism of adhesion of lymphocytes. Despite repeated attempts, no AF activity has been found (yet) from the dissociation of the thymus into single cells (Evans & Davies, unpublished observations). The specific ligand theory allows for a breaking-up of a stable adhesion, necessarily required for emigration from the node. Roseman's (1970) glycosyltransferase theory also allows for the breaking of the adhesive bond in certain conditions, which may be correlated with the rate of supply of the substrate. As mentioned, the specific ligand theory and the glycosyltransferase theories have much in common, but are they compatible with the IMF theory? IMF could block any of the enzyme activation or substrate supply requirements in these mechanisms but the fact that T IMF does not affect T cells could mean that the chemical systems of adhesion in T and B cells are not the same, although with enzyme specificities, they might be very similar. Even the DAH does not rule out the possibility of different adhesive mechanisms.

The IMF theory can thus explain the sorting-out of T and B cells within the lymph nodes (and Peyer's patches and the spleen), at least, consideration of the other theories does not disprove it. The DAH does show features that are consistent with observations but this theory has little experimental proof, whereas predictions from the IMF theory are more experimentally based. Unfortunately, the in vivo sorting-out in the node is not amenable to experimental analysis of the kind that is necessary to provide more evidence in favour of any of the theories put forward. Other than the IMF theory, none of the other theories has even considered lymphocytes (or indeed other mammalian cells), probably due to the greater complexities of the systems involved.

It is hoped that some of the results in this thesis have at least stimulated the idea of the importance of lymphocyte adhesive interactions in recirculation, but what of the future for interaction modulation factors? Perhaps the most important point to resolve is whether IMFs are normally produced *in vivo*. At this time, the production of IMFs *in vivo* has not been completely proved and before we can use the theory to postulate mechanisms for recirculation, the presence of IMFs *in vivo* must be confirmed. IMF-like activity has been detected in sera which are allogeneic or xenogeneic to the assay system, although these sera were from abnormal subjects. Detection of IMFs as defined by the work on syngeneic mice will require sophisticated biochemical techniques but before this can happen, the *in vitro*-produced IMFs must be thoroughly chemically characterised. However, as the results here have shown, it seems that the IMF preparations are not yet pure enough since different preparations produce different effects. This may have something to do with the stability of the substance, about which little also is known. Analysis of the sera of syngeneic athymic (nude) rats and mice may help in the search for *in vivo* IMFs since these animals should not possess T derived IMF. If IMFs are definitely found, then the suggested concepts about segregation in lymphoid organs can be pursued; until then, the actions of extraneously produced IMFs *in vivo* remain of academic interest only.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdou, N. L., Alavi, J. B. & Abdou, N. I. (1976)

Human bone marrow lymphocytes: B & T cell precursors and subpopulations. Blood 47, 423-430

Adler, W. J., Kong, Y. S. & Friedman, H. A. (1978)

Effect of cell-free murine liver extract on lymphocyte blastogenesis in vitro. Cell. Immunol. 36, 271-282.

Alexander, P., Sanders, S. & Braylan R. (1976)

Purported difference between human T- and B-cell surface morphology is an artefact. Nature (Lond.) 261, 239-241

Alexander, P. L. & Wetzel, B. (1975)

Human lymphocytes: Similarity of B & T cell surface morphology Science (N.Y.) 188, 732-734.

Anderson, A. O. & Anderson, N. D. (1975)

Studies on the structure and permeability of the microvasculature in normal rat lymph nodes. Am. J. Path., 80, 387-418

Anderson A. O.& Anderson, N. D. (1976)

Lymphocyte emigration from high endothelial venules in rat lymph nodes. Immunology 31, 731-748

Anderson, N. D., Anderson, A. O. & Wyllie, R. G. (1976)

Specialized structure and metabolic activities of high endothelial venules in rat lymphatic tissues. Immunology 31, 455-473

Arndt, R., Stark R., Klein, P. & Thiele, H.-G. (1978)
Serological and chromatographic analyses of antigenic structures detected in human brain, thymus and lymph nodes.
Immunology 35 95-103

Bach, J.-F. & Dardenne, M. (1973)
Studies on thymus products.
II Demonstration and characterization of a circulating thymic hormone. Immunology 25 353-366

Bach, J.-F., Dardenne, M., Goldstein, A. L., Guha, A. & White, A. (1971)
Appearance of T-cell markers in Bone Marrow Rosette-forming cells after incubation with thymosin, a thymic hormone.
Proc. Nat. Acad. Sci. U.S.A. 68 2734-2738.

Bach, J.-F., Dardenne, M. & Pleau, J.-M. (1977)
Biochemical characterization of a serum thymic factor
Nature (Lond.) 266 55-57

Bach, J.-F., Dardenne, M., Pleau, J.-M. & Bach, M.-A. (1975)
Isolation, biochemical characteristics and biological activity of a circulating thymic hormone in the mouse and in the human.
Ann. N.Y. Acad. Sci. 249 186-210

Bach, J.-F., Papiernik, M., Levasseur, P., Dardenne, M., Barois, A., & Le Brigand, H. (1972)
Evidence for a serum-factor secreted by the human thymus
Lancet ii (1972) 1056-1058

Bainbridge, D. R., Brent, L. & Gowland, G. (1966)

Distribution of allogeneic ^{51}Cr- labelled lymph node cells in mice. Transplantation 4 138-153.

Bainbridge, D. R. & Gowland, G. (1966)

Detection of homograft sensitivity in mice by the elimination of chromium-51 labelled lymph node cells. Ann. N.Y. Acad. Sci. 129 257-273

Barg, M. & Draper, L. R. (1975)

Migration of thymus cells to the developing gut-associated lymphoid tissues of the young rabbit. Cell. Immunol. 20 177-186

Barnetson, R. St. C., Bjune, G. & Duncan, M. E. (1976)

Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to Mycobacterium leprae. Nature (Lond.) 260 150-151

Barth, R. F. & Singla, O. (1975)

Migratory patterns of Tc-99m-labelled lymphoid cells. Effects of ALS on organ distribution of murine thymocytes. Cell Immunol. 17 83-95

Basten, A., Miller, J. F. A. P., Warner, N. L. & Pye, J. (1971)

Specific inactivation of thymus-derived (T) and non-thymus derived (B) lymphocytes by ^{125}I-labelled antigen. Nature New Biology 231 104-106

Basten, A., Sprent, J. & Miller, J. F. A. P. (1972)

Receptor for antibody-antigen complexes used to separate T cells from B cells. Nature New Biology 235 178-180.

Beamish, M. R., Ashley-Jones, P., Trevett, D., Howell Evans, I. & Jacobs, A. (1972)

Iron metabolism in Hodgkin's disease. Brit. J. Cancer 26 444-452

Bell, E. B. & Shand, F. L. (1975)

Changes in lymphocyte recirculation and liberation of the adoptive memory response from cellular-regulation in irradiated recipients. Eur. J. Immunol. 5 1-7

Benner, R., van Oudenaren, A. & De Ruiter, H. (1977)

B memory cells in the thymus: part of the pool of potentially circulating memory cells. J. Immunol. 119 1846-1848

Berney, S. N. & Gesner, B. M. (1970)

The circulatory behaviour of normal and enzyme altered thymocytes in rats. Immunology 18 681-691

Bianco, C., Patrick, R. & Nussenzweig, V. (1970)

A population of lymphocytes bearing a membrane receptor for antigen-antibody complexes. I. Separation and Characterisation. J. exp. med. 132 702-720.

Bieber, C. P. & Bieber, M. M. (1973)

Detection of ferritin as a circulating tumour associated antigen in Hodgkin's disease. Nat. Cancer Inst. Monographs 36 147-153.

Bienenstock, J., Johnston, N. & Perey, D. Y. E. (1973)

Bronchial lymphoid tissue. 1. Morphologic characteristics. Lab. Invest. 28 686-692

Binns, R. M. (1973)

Cellular immunology in the pig. Proc. Roy. Soc. Med. 66 1155-116

Binns, R. M. & Hall, J. G. (1966)

The paucity of lymphocytes in the lymph of unanaesthetised pigs. Brit. J. Exp. Path. 47 275-280

Blankwater, M.-J., Levert, L. A., Swart, A. C. W. & van Bekkum, D. W. (1978)

Effect of various thymic and non thymic factors on in vitro antibody formation by spleen cells from nude mice. Cell. Immunol. 35 242-252

Blomgren, H. & Andersson, B. (1969)

Evidence for a small pool of immunocompetent cells in the mouse thymus. Exp. Cell Res. 57 185-192

Blomgren, H. & Andersson, B. (1971)

Characteristics of the immunocompetent cells in the mouse thymus: cell population changes during cortisone-induced atrophy and subsequent regeneration. Cell. Immunol. 1 545-560

Blomgren, H. & Andersson, B. (1972)

Recirculating lymphocytes in the mouse thymus are part of the relatively cortisone-resistant cell population. Clin. Exp. Immunol. 10 297-303

Boak, J. L. & Woodruff, M. F. A. (1965)

A modified technique for collecting mouse thoracic duct lymph Nature (Lond.) 205 396-397.

von Boehmer, H., Shortman, K. & Nossal, G. J. V. (1974)

The separation of different cell classes from lymphoid organs.

X. Preparative electrophoretic separation of lymphocyte sub-populations from mouse spleen and thoracic duct lymph. J. Cell. physiol. 83 231-242

de Bono, D. (1976)

Endothelial-lymphocyte interactions in vitro. I. Adherence of non-allergised lymphocytes. Cell Immunol. 26 78-88

Boyle, W. (1968)

An extension of the ^{51}Cr-release assay for estimation of mouse cytotoxins. Transplantation 6 761-764.

Bøyum, A. (1968)

Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 21 suppl. 97, page 1.

Bradfield, J. W. B. & Born, G. V. R. (1969)

Inhibition of lymphocyte recirculation by heparin. Nature (Lond.) 222 1183-1184.

Bradfield, J. W. B. & Born, G. V. R. (1973)

The migration of rat thoracic duct lymphocytes through the spleen in vivo. Brit. J. Exp. Path. 54 509-517.

Bradfield, J. W. B. & Born, G. V. R. (1974)

Lymphocytosis produced by heparin and other sulphated polysaccharides in mice and rats. Cell Immunol. 14 22-32

Bremerskov, V. (1973)

Dextran sulphate inhibits cell adhesion in tissue culture. Nature New Biol. 246 174P.

Brooks, D. E. & Seaman, G. V. F. (1973)

The effect of neutral polymers on the electrokinetic potential of cells and other charged particles.

I. Models for the Zeta potential increase. J. Coll. interface sci. 43 670-686

Bryant, B. J. (1971)

Quantitative studies of thymokinetics.

From "Morphological and functional aspects of Immunity".

eds. K. Lindahl-Kiessling, G. Alm & M. G. Hanna.

Plenum Press, New York. pp. 103-112

Burdick, M. L. (1968)

A test of the capacity of chick embryo cells to home after vascular dissemination. J. Exp. Zool. 167 1-20

Burke, T. R. & Ambrus, J. L. (1976)

Induction of Theta-positive murine lymphocytes by a thymic extract. Res. Commun. Chem. Path. Pharm. 14 349-366

Burnet, F. M. (1959)

Clonal selection theory of acquired immunity. ix + 209 pages.

Cambridge University Press.

Cahill, R. N. P., Poskitt, D. C., Frost, H. & Trnka, Z. (1977)

Two distinct pools of recirculating T lymphocytes: migratory characteristics of nodal and intestinal T lymphocytes. J. exp. med. 145 420-428

Campbell, P. A. & Grey, H. M. (1972)

Removal of immunoglobulin-bearing lymphocytes by anti-immunoglobulin coated columns. Cell. Immunol. 5 171-179

Cantor, H. & Boyse, E. A. (1975)

Functional subclasses of T lymphocytes bearing different Ly antigens.

I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen.

J. exp. med. 141 1376-1389

Cantor, H., Simpson, E., Sato, V. L., Fathman, C. G. & Herzenberg, L. A. (1975)

Characterization of sub-populations of T lymphocytes.

I. Separation and functional studies of peripheral T-cells binding different amounts of fluorescent anti-Thy 1.2 (Theta) antibody using a fluorescence-activated cell sorter (FACS).

Cell. Immunol. 15 180-196

Casali, P. & Perussia, B. M. (1977)

C-3 reacted Sepharose: a preparative method for separating T and B lymphocytes. Clin. Exp. Immunol. 27 38-42

Cauldwell, C. B., Henkart, P. & Humphreys, T. (1973)

Physical properties of sponge aggregation factor. A unique proteoglycan complex. Biochemistry 12 3051-3055

Chess, L., MacDermott, R. P. & Schlossman, S. F. (1974)

Immunologic functions of isolated human lymphocyte subpopulations.

1. Quantitative isolation of human T and B cells and response to mitogens. J. Immunol. 113 1113-1121.

Chibon, P. (1967)

Marquage nucléaire par la thymidine tritiée des dérivés de la crête neurale chez L'Amphibien Urodèle Pleurodeles waltlii Michah. J. embryol. exp. morph. 18 343-358

Cochran, W. G. (1964)

Approximate significance levels of Behrens-Fisher test. Biometrics 20 191-195

Curtis, A. S. G. (1969)

The measurement of cell adhesiveness by an absolute method. J. embryol. exp. morph. 23 305-325

Curtis, A. S. G. (1973)

Cell adhesion. Prog. Biophys. Mol. Biol. 27 315-386

Curtis, A. S. G. (1978a)

Cell-cell recognition: positioning and patterning systems. Symp. Soc. Exp. Biol. 32 52-82

Curtis, A. S. G. (1978b)

Cell positioning.

From "Receptors & Recognition" Series B. Vol. 4

Specificity of embryological interactions.

ed. D. R. Garrod. Chapman & Hall, London. pp. 159-195

Curtis, A. S. G., Campbell, J. & Shaw, F. M. (1975a)

Cell surface lipids and adhesion.

I. The effects of lysophosphatidyl compounds, phospholipase A_2 and aggregation-inhibiting protein. J. Cell. Sci. 18 347-356

Curtis, A. S. G., Davies, M. & Wilkinson, P. C. (1978)

New evidence on control mechanisms in lymphocyte traffic.

To be published in the "Proceedings of the 6th International Conference on lymphatic tissues and Germinal Centres in Immune reactions", Plenum Press, New York.

Curtis, A. S. G. & Greaves, M. F. (1965)

The inhibition of cell aggregation by a pure serum protein.

J. embryol. exp. morph. 13 309-326

Curtis, A. S. G., Shaw, F. M. & Spires, V. M. C. (1975b)

Cell surface lipids and adhesion.

II. The turnover of lipid components of the plasmalemma in relation to cell adhesion. J. Cell. Sci. 18 357-373

Curtis, A. S. G. & de Sousa, M. A. B. (1973)

Factors influencing adhesion of lymphoid cells.

Nature New Biology 244 45-47

Curtis, A. S. G. & de Sousa, M. A. B. (1975)

Lymphocyte interactions and positioning. I. Adhesive interactions

Cell. Immunol. 19 282-297

Curtis, A. S. G. & van de Vyver, G. (1971)

The control of cell adhesion in a morphogenetic system.

J. embryol. exp. morph. 26 295-312

Danielle, R. P., Beacham, C. H. & Gorenberg, D. J. (1977)

The Bronchoalveolar lymphocyte.

Studies on the life history and lymphocyte traffic from blood to the lung. Cell. Immunol. 31 48-54.

Dardenne, M., Papiernik, M., Bach, J.-F. & Stutman, O. (1974)
Studies on thymus products. III. Epithelial origin of the serum thymic factor. Immunology 27 299-304

Davie, E. W., Fujikawa, K., Legaz, M. E. & Kato, H. (1975)
Role of proteases in blood coagulation
From "Proteases and Biological control"
Cold Spring Harbor Conferences on Cell proliferation Vol. 2
eds. K. Reich, D. B. Rifkin & E. Shaw. pp. 65-77

Despont, J. P., Abel, C. A. & Grey, H. M. (1975)
Sialic acid and sialyltransferases in murine lymphoid cells - indicators of T cell maturation. Cell. Immunol. 17 487-494

Diamantstein, T., Vogt, W., Ruhl, H. & Bochert, G. (1973)
Stimulation of DNA synthesis in mouse lymphoid cells by polyanions in vitro. 1. Target cells and possible mode of action.
Eur. J. Immunol. 3 488-493

Dietrich, C. P., Sampaio, L. O., Toledo, O. M. S. & Cassaro, C. M.F. (1977)
Cell recognition and adhesiveness: a possible biological role for the sulfated mucopolysaccharides. Biochem. Biophys. Res. Commun. 75 329-336

Durkin, H. G., Theis, G. A. & Thorbecke, G. J. (1971)
Homing of cells from the Bursa of Fabricius to germinal centres in the chicken spleen. From "Morphological and functional aspects of Immunity" eds. K. Lindahl-Kiessling, G. Alm & M. G. Hanna. Plenum Press, New York. pp. 119-128

Edelstein, B. B. (1971)

Cell specific diffusion model of morphogenesis

J. theor. biol. 30 515-532

Ernstrom, U. & Nordlind, K. (1977)

Thymic factors regulating DNA synthesis in lymphocytes cultured in vitro. Int. Archs. Allergy Appl. Immunol. 54 463-472

Evans, C. W. & Davies, M. E. J. (1977)

The influence of cell adhesiveness on the migratory behaviour of murine thymocytes. Cell Immunol. 33 211-218

van Ewijk, W., Brons, N. H. C. & Rozing, J. (1975)

Scanning electron microscopy of homing and recirculating lymphocyte populations. Cell. Immunol. 19 245-261

Faulk, W. P., McCormick, J. N., Goodman, J. R., Yoffey, J. M. & Fudenberg, H. H. (1971)

Peyer's patches: morphologic studies. Cell. Immunol. 1 500-520

Ford, W. L. (1968)

The mechanism of lymphopenia produced by chronic irradiation of rat spleen. Brit. J. Exp. Path. 49 502-510

Ford, W.L. (1969)

The kinetics of lymphocyte recirculation within the rat spleen.

Cell Tissue Kinet. 2 171-191

Ford, W. L. (1975)

Lymphocyte migration and immune response.

Prog. Allergy 19 1-59

Ford, W. L. (1978)

The preparation and labelling of lymphocytes

From "Handbook of Experimental Immunology" ed. D. M. Weir

3rd edition. Blackwell Scientific Publications, Oxford

Volume 2, Chapter 23, pp. 231- 23.22

Ford, W. L. & Hunt, S. V. (1973)

The preparation and labelling of lymphocytes

From "Handbook of Experimental Immunology" ed. D. M. Weir

2nd edition. Blackwell Scientific Publications, Oxford

Volume 2, Chapter 23, pp. 23.1-23.27.

Ford, W. L., Sedgley, M., Sparshott, S. M. & Smith, M. E. (1976)

The migration of lymphocytes across specialised vascular endothelium. II. The contrasting consequences of treating lymphocytes with trypsin or neuraminidase. Cell Tissue Kinet. 9 351-361

Ford, W. L., Smith, M. E. & Andrews, P. (1978)

Possible clues to the mechanism underlying the selective migration of lymphocytes from the blood. Symp. Soc. Exp. Biol. 32 359-392

Freitas, V. A., Rocha, B., Chiao, J. W. & de Sousa, M. (1978)

Divergent actions of monomeric, dimeric and tetrameric Con A on lymphocyte traffic. Cell Immunol. 35 59-77

de Freitas, V. A., Rose, M. L. & Parrott, D. M. V. (1977)

Murine mesenteric and peripheral lymph nodes: a common pool of small T cells. Nature (Lond.) 270 731-733

Freitas, A. A., & de Sousa, M. (1975)

Control mechanisms of lymphocyte traffic. Modification of the traffic of ^{51}Cr labelled mouse lymph node cells by treatment with plant lectins in intact and splenectomised hosts. Eur. J. Immunol. 5 831-838

Freitas, A. A. & de Sousa, M. (1976a)

The role of cell interactions in the control of lymphocyte traffic Cell. Immunol. 22 345-350

Freitas, A. A. & de Sousa, M. (1976b)

Control mechanisms of lymphocyte traffic. Altered distribution of ^{51}Cr-labelled mouse lymph node cells pretreated in vitro with lipopolysaccharide. Eur. J. Immunol. 6 269-273

Freitas, A. A. & de Sousa, M. (1976c)

Control mechanisms of lymphocyte traffic. Altered migration of ^{51}Cr-labelled mouse lymph node cells pretreated in vitro with phospholipases. Eur. J. Immunol. 6 703-711

Freitas, A. A. & de Sousa, M. (1977)

Control mechanisms of lymphocyte traffic. A study of the action of two sulphated polysaccharides on the distribution of ^{51}Cr- and ^{3}H adenosine-labelled mouse lymph node cells. Cell. Immunol. 31 62-76

Friedman, H. (1975) Editor

Thymus factors in Immunity. Ann. N. Y. Acad. Sci. 249

Frost, H., Cahill, R. N. P. & Trnka, Z. (1975)

The migration of recirculating autologous and allogeneic lymphocytes through single lymph nodes. Eur. J. Immunol. 5 839-843

Frost, P. & Lance, E. M. (1974)

The cellular origin of the lymphocyte trap.

Immunology 26 175-186

Garaci, E., del Gobbo, V. & Rinaldi-Garaci, C. (1976)

Effects of antithymic reticuloepithelial cells serum on the levels of circulating thymic factor and on the sensitivity to azathioprine of spleen spontaneous rosette-forming cells. Experientia 32 1475-1476

Gasic, G. & Gasic, T. (1962)

Removal of sialic acid from the cell coat in tumour cells and vascular endothelium and its effects on metastasis.

Proc. Nat. Acad. Sci. U.S.A. 48 1172-1177

Gery, I., Navok, T. & Stupp, Y. (1977)

Selective Accumulation of cells with 'B' properties in stimulated lymph nodes. Immunology 33 727-731

Gesner, B. M. & Ginsburg, V. (1964)

Effect of glycosidases on the fate of transfused lymphocytes.

Proc. Nat. Acad. Sci. U.S.A. 52 750-755

Gesner, B. M. & Gowans, J. L. (1962)

The output of lymphocytes from the thoracic duct of unanaesthetised mice. Brit. J. Exp. Path. 43 424-430

Gesner, B. M., Woodruff, J. J. & McCluskey, R. T. (1969)

An autoradiographic study of the effect of neuraminidase or trypsin on transfused lymphocytes. Am. J. Path. 57 215-230

Gillette, R. W., McKenzie, G. O. & Swanson, M. H. (1973)

Effect of concanavalin A on the homing of labelled T lymphocytes J. Immunol. 111 1902-1905

Goldschneider, I. (1976)

Antigenic relationship between bone marrow lymphocytes, cortical thymocytes and a subpopulation of peripheral T cells in the rat: description of a bone marrow lymphocyte antigen. Cell Immunol. 24 289-307

Goldschneider, I. & McGregor, D. D. (1968)

Migration of lymphocytes and thymocytes in the rat.

I. Route of migration from blood to spleen and lymph node.

J. exp. med. 127 155-168

Goldstein, A. L. Guha, A., Zatz, M. M., Hardy, M. A. & White, A. (1972)

Purification and biological activity of thymosin, a hormone of the thymus gland. Proc. Nat. Acad. Sci. U.S.A. 69 1800-1803

Goldstein, G. (1968)

The thymus and neuromuscular function. A substance in thymus which causes myositis and myasthenic neuromuscular block in guinea pigs. Lancet ii (1968) 119-122

Goldstein, G. (1974)

Isolation of bovine thymin: a polypeptide hormone of the thymus Nature (Lond.) 247 11-14

Goldstein, G. (1975)

The isolation of thymopoietin (thymin)

Ann. N. Y. Acad. Sci. 249 177-185

Goos, M., Kaiserling, E. & Lennert, K. (1976)

Mycosis fungoides: model for T-lymphocyte homing to the skin.

Brit. J. Dermat. 94 221-222

Götze, O. (1975)

Proteases of the properdin system.

From "Proteases and biological control"

Cold Spring Harbor Conference on cell proliferation. Vol. 2.

eds. K. Reich, D. B. Rifkin & E. Shaw. pp. 255-272

Gowans, J. L. (1959)

The recirculation of lymphocytes from blood to lymph in the rat.

J. physiol. 146 54-69

Gowans, J. L. & Knight, E. J. (1964)

The route of recirculation of lymphocytes in the rat.

Proc. Roy. Soc. Lond. Ser. B. 159 257-282

Grant, L. (1973)

The sticking and emigration of white blood cells in inflammation.

From "The inflammatory Process" 2nd edition. eds. B. W.

Zweifach, L. Grant & R. T. McCluskey, Academic Press,

New York. pp. 205-249.

Greaves, M. F. & Brown, G. (1974)

Purification of human T and B lymphocytes. J. Immunol. 112

420-423.

Greaves, M. F., Owen, J. J. T. & Raff, M. C. (1974)

T & B lymphocytes. Origins, properties and roles in immune responses. vii + 316 pages. Excerpta Medica, Amsterdam.

Gutman, G. A. & Weissman, I. L. (1973)

Homing properties of T-independent folicular lymphocytes Transplantation 16 621-629

Hall, J. G. & Morris, B. (1965)

The origin of the cells in the efferent lymph from a single lymph node. J. exp. med. 121 901-910

Handwerger, B. S. & Schwartz, R. H. (1974)

Separation of murine lymphoid cells using nylon wool columns. Transplantation 18 544-548

Harris, J. E., Ford, C. E., Barnes, D. W. H. & Evans, E. P. (1964)

Evidence from parabiosis for an afferent stream of cells. Nature (Lond.) 201 886-887

Harris, R. & Ukaejiofo, E. O. (1970)

Tissue typing using a routine one-step lymphocyte separation procedure. Brit. J. Haematol. 18 229-235

Hay, J. B. & Hobbs, B. B. (1977)

The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. J. exp. med. 145 31-44

Herscowitz, H. B. & McKillip, T. W. (1974)

A simple method for liquid scintillation counting of ^{125}I and ^{51}Cr used in antigen binding and cytotoxicity studies. J. Immunol. methods 4 253-262

Herscowitz, H. B. & McKillip, T. W. (1974)

A simple method for liquid scintillation counting of ^{125}I and ^{51}Cr used in antigen binding and cytotoxicity studies.

J. immunol. methods 4 253-262

Hersey, P. (1971)

The separation and ^{51}Chromium labelling of human lymphocytes with in vivo studies of survival and migration. Blood 38 360-371

Hollingsworth, J. W. & Carr, J. (1972)

Migration of rat thoracic duct lymphocytes from tissues to central lymphatic circulation. Cell. Immunol. 5 228-232

Hooper, J. A., McDaniel, M. C., Thurman, G. B., Cohen, G. H. Schulof, R. S. & Goldstein, A. L. (1975)

Purification and properties of bovine thymosin.

Ann. N. Y. Acad. Sci. 249 125-144

Howard, J. C. (1972a)

The life-span and recirculation of marrow-derived small lymphocytes from the rat thoracic duct. J. exp. med. 135 185-199

Howard, J. C., Hunt, S. V. & Gowans, J. L. (1972b)

Identification of marrow-derived and thymus-derived small lymphocytes in the lymphoid tissue and thoracic duct lymph of normal rats. J. exp. med. 135 200-219

Huber, B., Cantor, H., Shen, F. W. & Boyse, E. A. (1976)

Independent differentiative pathways of Ly1 and Ly23 subclasses of T cells. Experimental production of mice deprived of selected T cell subclasses. J. exp. med. 144 1128-1133

Hudson, G. & Yoffey, J. M. (1966)

The passage of lymphocytes through the sinusoidal endothelium of guinea-pig bone marrow. Proc. Roy. Soc. Lond, Ser. B 165 486-496

Hudson, L. & Hay, F. C. (1976)

Practical Immunology xvi + 298 pages

Blackwell Scientific Publications, Oxford

Humphreys, T. (1963)

Chemical dissolution and in vitro reconstruction of sponge cell adhesion: I. Isolation and functional demonstration of the components involved. Develop. Biol. 8 27-47

Hunt, S. V. (1978)

Separation of lymphocyte sub-populations

From "Handbook of Experimental Immunology" ed. D. M. Weir 3rd edition. Blackwell Scientific Publications, Oxford. Volume 2 Chapter 24, pp. 24.1-24.26

Hunt, S. V., Mason, D. W. & Williams, A. F. (1977)

In rat bone marrow Thy-1 antigen is present on cells with membrane immunoglobulin and on precursors of peripheral B lymphocytes

Eur. J. Immunol. 7 817-823

Irlé, C., Piguet, P.-F. & Vassalli, P. (1978)

In vitro maturation of immature thymocytes into immunocompetent T cells in the absence of direct thymic influence.

J. exp. med. 148 32-45

Jancik, J. M., Schauer, R., Andres, K. H. & von Düring, M. (1978)
Sequestration of neuraminidase-treated erythrocytes. Studies on its topographic, morphologic and immunologic effects. Cell. Tiss. Res. 186 209-226

Jansen, C. R., Cronkite, E. P., Mather, G. C., Nielson, N. O., Rai, K., Adamik, E. R. & Sipe, C. R. (1962)
Studies on lymphocytes. II The production of lymphocytosis by intravenous heparin in calves. Blood 20 443-452

Joel, D. D., Chanana, A. D., Cottier, H., Cronkite, E. P. & Laissue, J. A. (1977)
Fate of thymocytes - studies with ^{125}I-iododeoxyuridine and ^{3}H-thymidine in mice. Cell. Tiss. Kinet. 10 57-69

Joel, D. D., Hess, M. W. & Cottier, H. (1971)
Thymic origin of lymphocytes in developing Peyer's patches of newborn mice. Nature New Biology 231 24-25

Julius, M. H., Simpson, E. & Herzenberg, L. A. (1973)
A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3 645-649

Kadish, J. L. & Basch, R. S. (1977)
Hematopoietic thymocyte precursors. III. A population of thymocytes with the capacity to return ("home") to the thymus. Cell. Immunol. 30 12-24

Kaliss, N. & Pressman, D. (1950)
Plasma and blood volumes of mouse organs, as determined with radioactive iodoproteins. Proc. Soc. Exp. Biol. Med. 75 16-20

Karpf, M., Gelfand, M. C., Handwerger, B. S. & Schwartz, R. H. (1975)

Lack of B lymphocyte depletion from murine spleen cell populations by a human gamma-globulin, anti-human gamma-globulin system. J. immunol. 114 542-545

Kasakura, S. (1977)

Blastogenic factors: production by T and B lymphocytes Immunology 33 23-29

Kiger, N., Florentin, I., Lorans, G. & Mathe, G. (1977)

Further purification and chemical characterisation of the lymphocyte-inhibiting-factor extracted from the thymus. Immunology 33 439-448

Kook, A. I. & Trainin, N. (1974)

Hormone-like activity of a thymus humoral factor on the induction of immune competence in lymphoid cells. J. exp. med. 139 193-207

Kruisbeek, A. M., Astaldi, T. C. B., Blankwater, M.-J., Zijlstra, J.J. Levert, L. V. & Astaldi, A. (1978)

The in vitro effect of a thymic epithelial culture supernatant on mixed lymphocyte reactivity and intracellular cyclic AMP levels of thymocytes and on antibody production to SRBC by nu/nu spleen cells. Cell. Immunol. 35 134-147

Kruisbeek, A. M., Kröse, T. C. J. M. & Zijlstra, J. J. (1977)

Increase in T cell mitogen responsiveness in rat thymocytes by thymic epithelial culture supernatant. Eur. J. Immunol. 7 375-381

De Kruyff, R. H., Gilmour, D. G. & Thorbecke, G. J. (1975)
Migratory patterns of B lymphocytes. III. Inhibition of splenic follicular localisation of transferred chicken bursa cells by preincubation with anti-Ig. J. Immunol. 114 1700-1704

Laissue, J. A., Chanana, A. D., Cottier, H., Cronkite, E. P. & Joel, D. D. (1976)
Fate of intraintestinal thymocytes labelled with ^{125}Iododeoxyuridine or tritiated thymidine. Proc. Soc. Exp. Biol. Med. 152 262-265

Letarte-Muirhead, M., Barclay, A. N., & Williams, A. F. (1975)
Purification of the Thy 1 molecule, a major cell-surface glycoprotein of rat thymocytes. Biochem. J. 151 685-697

Lilien, J., Hermolin, J. & Lipke, P. (1978)
Molecular interactions in specific cell adhesion.
From "Receptors & Recognition" Series B. Vol. 4
Specificity of embryological interactions. ed. D. R. Garrod.
Chapman & Hall, London. pp. 133-155

Lloyd, C. W. & Cook, C. M. W. (1974)
On the mechanism of increased aggregation by neuraminidase of 16c malignant rat dermal fibroblasts in vitro.
J. Cell Sci. 15 575-590

Loor, F. & Hägg, L.-B. (1975)
The modulation of microprojections on the lymphocyte membrane and the redistribution of membrane-bound ligands, a correlation.
Eur. J. Immunol. 5 854-865.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951)

Protein measurement with the Folin phenol reagent.

J. biol. chem. 193 265-275

Malyshev, V. A. (1977)

The effect of a low molecular humoral factor of the thymus on the susceptibility of lymphocytes to phytohaemagglutinin. (Russian text, English summary). Cytologia 19 832-836

Mandi, B. & Glant, T. (1973)

Thymosin-producing cells of the thymus. Nature New Biology 246 25P

Marchesi, V. T. & Gowans, J. L. (1964)

The migration of lymphocytes through the endothelium of venules in lymph nodes: an electron microscope study.

Proc. Roy. Soc. Lond. Ser. B. 159 282-290

Martin, W. J. (1969)

Assay for the immunosuppressive capacity of anti-lymphocyte serum. I. Evidence for opsonisation. J. immunol. 103 979-999

Mehrishi, J. N. & Zeiller, K. (1974)

Surface molecular components of T and B lymphocytes.

Eur. J. Immunol. 4 474-478

Metcalf, D. & Wiadrowski, M. (1966)

Autoradiographic analysis of lymphocyte proliferation in the thymus and in thymic lymphoma tissue. Cancer Res. 26 483-491

Meyer, D. B. (1964)

The migration of primordial germ cells in the chick embryo.

Develop. biol. 10 154-190

Mitchell, J. (1972)

Antigens in immunity. XVII. The migration of antigen-binding, bone-marrow-derived and thymus-derived spleen cells in mice.

Immunology 22 231-245

Moore, M. A. S. & Owen, J. J. T. (1966)

Experimental studies on the development of the bursa of Fabricius

Develop. biol. 14 40-51

Moroz, C., Giller, S., Kupfer, B. & Urca, I. (1977)

Lymphocytes bearing surface ferritin in patients with Hodgkin's disease and breast cancer. New Eng. J. Med. 296 1172-1173

Morrison, J. H. (1967)

Separation of lymphocytes of rat bone marrow by combined glass-wool filtration and dextran-gradient centrifugation.

Brit. J. Haematol. 13 229-235

Morse, S. I. & Barron, B. A. (1970)

Studies on the leucocytosis and lymphocytosis induced by Bordetella pertussis. III. The distribution of transferred lymphocytes in pertussis-treated and normal mice. J. exp. med. 132 663-677

Morse, S. I. & Morse, J. H. (1976)

Isolation and properties of the leucocytosis and lymphocytosis-promoting factor of Bordetella pertussis. J. exp. med. 143 1483-1502

Newell, D. G., Roath, S. & Smith, J. L. (1976)

The scanning electron microscopy of normal human peripheral blood lymphocytes. Brit. J. Haematol. 32 304-316

Nicolson, G. L. (1972)

Topography of membrane Con A sites modified by proteolysis. Nature New Biology 239 193-197

Nieuwenhuis, P. (1971)

The rabbit appendix: a central or peripheral lymphoid organ? From "Morphological and functional aspects of immunity" eds. K. Lindahl-Kiessling, G. Alm & M. G. Hanna Plenum Press, New York pp. 25-30

Nieuwenhuis, P. & Ford, W. L. (1976)

Comparative migration of B- and T-lymphocytes in the rat spleen and lymph nodes. Cell. Immunol. 23 254-267

Nordling, S., Andersson, L. C. & Häyry, P. (1972)

Separation of T and B lymphocytes by preparative cell electrophoresis. Eur. J. Immunol. 2 405-410

Osmond, D. G., Wherry, P. F., Daeron, M. & Gordon, J. (1976)

Immunoglobulin-bearing cells in bone marrow of mice after prolonged treatment with antiIgM antibody. Nature (Lond.) 260 328-329

Osoba, D. & Miller, J. F. A. P. (1963)

Evidence for a humoral thymus factor responsible for the maturation of immunological faculty. Nature (Lond.) 199 653-654

Owen, J. J. T., Wright, D. E., Habu, S., Raff, M. C. & Cooper, M. D. (1977)

Studies on the generation of B lymphocytes in foetal liver and bone marrow. J. Immunol. 118 2067-2072

Pabst, R., Munz, D. & Trepel, F. (1977)

Splenic lymphocytopoiesis and migration pattern of splenic lymphocytes. Cell. Immunol. 33 33-44

Pabst, R. & Trepel, F. (1975)

The predominant role of the spleen in lymphocyte recirculation. I. Homing of lymphocytes to and release from the isolated perfused pig spleen. Cell Tissue Kinet. 8 529-541

Pabst, R. & Trepel, F. (1976)

The predominant role of the spleen in lymphocyte recirculation. II. Pre- and post-splenectomy retransfusion studies in young pigs. Cell Tissue Kinet. 9 179-189

Padnos, M. (1976)

Differentiation of B and T mouse lymphocytes in cell suspension and smears. Nature (Lond.) 259 218-220

Papiernik, M., Laroche, L. & Bach, J.-F. (1977)

Thymocyte subpopulations in young and adult mice. I. Separation by density gradient and steroid treatment. Eur. J. Immunol. 7 796-799

Parrott, D. M. V. & de Sousa, M. (1971)

Thymus-dependent and thymus-independent populations: origin, migratory patterns and lifespan. Clin. Exp. Immunol. 8 663-684

Pearson, L. D., Simpson-Morgan, M. W. & Morris, B. (1976)
Lymphopoisis and lymphocyte recirculation in the sheep fetus
J. exp. med. 143 167-186

Perey, D. Y. E., Cooper, M. D. & Good, R. A. (1968)
Lymphoepithelial tissue of the intestine and differentiation of antibody production. Science (N.Y.) 161 265-266

Perey, D. Y. E. & Milne, R. W. (1975)
Rabbit gut-associated lymphoid tissue. Major pathway for thoracic duct lymphocyte circulation. Lab. Invest. 33 678-686

Phillips, J. L. (1976)
Specific binding of zinc transferrin to human lymphocytes.
Biochem. Biophys. Res. Commun. 72 634-639

Polliack, A., Hämmerling, J., Lampen, N. & de Harven, E. (1975)
Surface morphology of murine B & T lymphocytes: a comparative study by scanning electron microscopy. Eur. J. Immunol. 5 32-39

Polliack, A., Lampen, N., Clarkson, B. D. & de Harven, E. (1973)
Identification of human B and T lymphocytes by scanning electron microscopy. J. exp. med. 138 602-624

Raff, M. C. (1969)
Theta isoantigen as a marker of thymus-derived lymphocytes in mice. Nature (Lond.) 224 378-379

Raff, M. C. (1971)
Evidence for a subpopulation of mature lymphocytes within mouse thymus. Nature New Biology 229 182-183

Raff, M. C. & Owen, J. J. T. (1971)

The use of surface alloantigenic markers to study the differentiation of thymus-derived lymphocytes in mice.

From "Morphological and functional aspects of immunity"

eds. K. Lindahl-Kiessling, G. Alm & M. G. Hanna,

Plenum Press, New York pp. 11-15

Raidt, D. J., Mishell, R. I. & Dutton, R. W. (1968)

Cellular events in the immune response.

Analysis and in vitro response of mouse spleen cell populations separated by differential flotation in albumin gradients.

J. exp. med. 128 681-698

Rannie, G. H. & Donald, K. J. (1977)

Estimation of the migration of thoracic duct lymphocytes to non-lymphoid tissues. A comparison of the distribution of radioactivity at intervals following intravenous transfusion of cells labelled with ^{3}H, ^{14}C, ^{75}Se, ^{99m}Tc, ^{125}I and ^{51}Cr in the rat.

Cell Tissue Kinet. 10 523-541

Rannie, G. H., Smith, M. J. & Ford, W. L. (1977a)

Lymphocyte migration into cell-mediated immune lesions is inhibited by trypsin. Nature (Lond.) 267 520-522

Rannie, G. H., Thakur, M. L. & Ford, W. L. (1977b)

An experimental comparison of radioactive labels with potential application to lymphocyte migration studies in patients.

Clin. Exp. Immunol. 29 509-514

Reif, A. E. & Allen, J. M. V. (1964)

The AKR thymic antigen and its distribution in leukaemias and nervous tissue. J. exp. med. 120 413-433

Reisner, Y., Linker-Israeli, M. & Sharon, N. (1976)

Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. Cell. Immunol. 25 129-134

Rogers, A. W. (1969)

Techniques in autoradiography. xi + 338

Elsevier Publishing Company, Amsterdam.

Roitt, I. M., Greaves, M. F., Torrigiani, G., Brostoff, J. & Playfair, J. H. L. (1969)

The cellular basis of immunological responses.

Lancet ii (1969) 367-371

Rolstad, B. & Toogood, E. (1978)

Toxicity of $Na_2{}^{51}CrO_4$ when used to label rat lymphocytes

J. immunol. methods 21 271-276

Ronai, P. M. (1969)

The elution of ^{51}Cr from labelled leukocytes - a new theory.

Blood 33 408-413

van Rooijen, N. (1977)

Labelling of lymphocytes with various radioisotopes for in vivo tracer studies; a review. J. immunol. methods 15 267-277

Rose, M. L. & Micklem, H. S. (1976)

^{75}Se L-selenomethionine: a new isotopic marker for lymphocyte localisation studies. J. immunol. methods 9 281-296

Roseman, S. (1970)

The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. Chem. Phys. Lipids 5 270-297

Ruhenstroth-Bauer, G. & Lücke-Huhle, C. (1968)

Two populations of small lymphocytes. J. cell biol. 37 [illegible]

Ruuskanen, O. (1975)

Subpopulations of guinea pig thymocytes. Different distribution patterns of alkaline phosphatase positive and negative autologous thymocytes. Cell. Immunol. 15 246-254

Rydgren, L., Norberg, B., Hakansson, C. H., v. Mecklenburg, C. & Söderström, N. (1976a)

Lymphocyte locomotion. I. The initiation, velocity, pattern and path of locomotion in vitro. Lymphology _ 89-96

Rydgren, L., Norberg, B., v. Mecklenburg, C., Hakansson, C. H. & Söderström, N. (1976b)

Lymphocyte locomotion. II. The lymphocyte traffic over the post-capillary venules analysed by phase contrast microscopy of thin sections of rat lymph nodes. Lymphology _ 96-100

Sainte-Marie, G. (1975)

A critical analysis of the validity of the experimental basis of current concept of the mode of lymphocyte recirculation.

Bull. Inst. Pasteur 73 255-279

Sainte-Marie, G. & Peng, F. S. (1975a)

Migration of intramediastinally injected thymocytes in draining nodes. Ann. Immunol. (Inst. Pasteur) 126C 501-516

Sainte-Marie, G. & Peng, F. S. (1975b)

Migration into pseudo-follicles of draining lymph nodes of medullary small thymocytes injected in the mediastinal cavity.

Rev. Canad. Biol. 34 205-212

Sainte-Marie, G., Peng, F. S. & Denis, G. (1975)

A study of the mode of lymphocyte recirculation in the dog.

Ann. Immunol. (Inst. Pasteur) 126C 481-500

Sainte-Marie, G., Sin, Y. M. & Chan, C. (1967)

The diapedesis of lymphocytes through post-capillary venules of rat lymph nodes. Rev. Canad. Biol. 26 141-151

Sanderson, A. R. (1964)

Cytotoxic reactions of mouse isoantisera.

Brit. J. Exp. Path 45 398-408

Schlesinger, M. & Israël, . (1974)

The effect of lectins on the migration of lymphocytes in vivo

Cell. Immunol. 14 66-79

Schoefl, G. I. (1972)

The migration of lymphocytes across the vascular endothelium in lymphoid tissue. A re-examination. J. exp. med. 136 568-588

Scollay, R., Hall, J. G. & Orlans, E. (1976)

Studies on the lymphocytes of sheep. II. Some properties of cells in various compartments of the recirculating lymphocyte pool. Eur. J. Immunol. 6 121-125

Sedgley, M. & Ford, W. L. (1976)

The migration of lymphocytes across specialised vascular endothelium. Cell Tissue Kinet. 9 231-243

Sheppard, G. & Marlow, C. G. (1971)

The simultaneous measurement of ^{51}Cr and ^{14}C by liquid scintillation counting. Int. J. Appl. Radiat. Isotop. 22 125-127

Smith, J. B., Cunningham, A. J., Lafferty, K. J. & Morris, B. (1970)

The role of the lymphatic system and lymphoid cells in the establishment of immunological memory. Aust. J. exp. biol. med. sci. 48 57-70

van Snick, J. L. & Masson, P. L. (1976)

The binding of human lactoferrin to mouse peritoneal cells. J. exp. med. 144 1566-1580

de Somer, P., Denys, P. & Leyton, R. (1963)

Activity of a non-cellular calf thymus extract in normal and thymectomised mice. Life Sci. 11 810-819

Soppi, E., Ruuskanen, O., Eskola, J., Früki, J. & Kouvalainen, K. (1977)

Two sub-populations of guinea-pig thymocytes with different maturation stages. Immunology 33 343-350

de Sousa, M. (1971)

Kinetics of the distribution of thymus and marrow cells in peripheral lymphoid organs of the mouse-ecotaxis.

Clin. Exp. Immunol. 9 371-380

de Sousa, M. (1976)

Cell Traffic

From "Receptors & Recognition" Series A, Vol. 2

eds. P. Cuatrecasas and M. F. Greaves, Chapman & Hall, London

pp. 105-163

de Sousa, M. (1978)

Lymphoid cell positioning: a new proposal for the mechanism of control of lymphoid cell migration.

Symp. Soc. Exp. Biol. 32 393-409

de Sousa, M. & Haston, W. (1976)

Modulation of B-cell interactions by T-cells.

Nature (Lond.) 260 429-430

de Sousa, M. & Nishiya, K. (1978)

Inhibition of E-rosette formation by two iron salts.

Cell. Immunol. 38 203-208

de Sousa, M., Smithyman, A. & Tan, C. (1978)

Suggested models of ecotaxopathy in lymphoreticular malignancy.

Am. J. Path. 90 497-520

de Sousa, M., Yang, M., Lopez-Corrales, G., Tan, C., Hansen, J. A. Dupont, B. & Good, R. A. (1977)

Ecotaxis: the principles and its application to the study of Hodgkin's disease. Clin. Exp. Immunol. 27 143-151

Sprent, J. (1976)

Fate of H-2 activated T lymphocytes in syngeneic hosts. I. Fate in lymphoid tissues and intestine traced with ^{3}H-TdR, ^{125}IUDR and ^{51}Cr. Cell. Immunol. 21 278-302

Sprent, J. (1977)

Recirculating lymphocytes.

From "The lymphocyte: structure and function; part 1"

ed. J. J. Marchalouis.

Marcel Dekkar, New York & Basel. Chapter 2. pp. 43-112

Spry, C. J. F. (1972)

Inhibition of lymphocyte recirculation by stress and corticotropin.

Cell. Immunol. 4 86-92

Spry, C. J. F., Lane, J. T. & Vyakarnam, A. (1977)

The effects of complement activation by cobra venom factor on the migration of T and B lymphocytes into rat thoracic duct lymph. Immunology 32 947-954

Stamper, H. B. & Woodruff, J. J. (1976)

Lymphocyte homing into lymph nodes: in vitro demonstration of the selective affinity of recirculating lymphocytes for high-

endothelial venules. J. exp. med. 144 828-833

Stamper, H. B. & Woodruff, J. J. (1977)

An in vitro model of lymphocyte homing. I. Characterisation of the interaction between thoracic duct lymphocytes and specialised high-endothelial venules of lymph nodes. J. immunol. 119 772-780

Steinberg, M. S. (1970)

Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells.
J. exp. zool. 173 395-434

Steinberg, M. S. (1978)

Specific cell ligands and the differential adhesion hypothesis: How do they fit together?
From "Receptors & Recognition" Series B. Vol. 4
Specificity of embryological interactions
ed. D. R. Garrod. Chapman & Hall, London. pp. 99-130

Strober, S. & Dilley, J. (1973)

Biological characteristics of T and B memory lymphocytes in the rat. J. exp. med. 137 1275-1292

Stutman, O. (1976)

Migration of Yolk Sac Cells to thymus grafts: requirement of prior sojourn in bone marrow (or liver?) Ann. Immunol. (Inst. Pasteur) 127C 943-949

Stutman, O. & Good, R. A. (1971)

Immune competence of cells derived from hemopoietic liver after traffic to thymus.

From "Morphological and functional aspects of immunity"

eds. K. Lindahl-Kiessling, G. Alm & M. G. Hanna

Plenum Press, New York. pp. 129-137

Sundaresan, P., Sainis, K. B., Sundaram, K. & Phondke, G. P. (1975)

Differential concentration of surface sialic acid in human T and B lymphocytes. Ind. J. Exp. biol. 13 523-526

Taketa, S. T., Carsten, A. L., Cohn, S. H. & Bond, V. P. (1970)

Active bone marrow distribution in the monkey.

Life Sci. 9 Part 2 169-174

Taub, R. N., Rosett, W., Adler, A. & Morse, S. I. (1972)

Distribution of labelled lymph node cells in mice during the lymphocytosis induced by Bordetella pertussis.

J. exp. med. 136 1581-1593

Trainin, N. & Small, M. (1970)

Studies on some physicochemical properties of a thymus humoral factor conferring immunocompetence on lymphoid cells.

J. exp. med. 132 885-897

Trinkaus, J. P. (1969)

Cells into organs. The forces that shape the embryo. xvi + 237 pages. Prentice-Hall Inc., New Jersey.

Trizio, D. & Cudkowicz, G. (1974)

Separation of T and B lymphocytes by nylon wool columns:

evaluation of efficacy by functional assays in vivo.

J. immunol. 113 1093-1097

Tse, H. & Dutton, R. W. (1976)

Separation of helper and suppressor T lymphocytes on a Ficoll velocity sedimentation gradient.

J. exp. med. 143 1199-1210

Turk, J. L. & Poulter, L. W. (1972)

Selective depletion of lymphoid tissue by cyclophosphamide

Clin. Exp. Immunol. 10 285-298

Turner, R. S. & Burger, H. M. (1973)

Involvement of a carbohydrate group in the active site for surface guided reassociation of animal cells.

Nature (Lond.) 244 509-510

Vassar, P. S., Levy, E. M. & Brooks, D. E. (1976)

Studies on the electrophoretic separability of B and T human lymphocytes. Cell. Immunol. 21 257-271

Viklicky, V, Peknicova, J. & Polackova, M. (1976)

The significance of the cell surface for the homing affinity of injected lymphoid cells in mice. Folia Biologica (Prague) 22 159-168

Vischer, T. L. (1974)

Stimulation of mouse B lymphocytes by trypsin.

J. immunol. 113 58-62

Vuitton, D, Eloy, R., Gosse, F., Pousse, A. & Grenier, J. F. (1977)
Subpopulations of T-lymphocytes in Peyer's patches: sensitivity to anti-lymphocyte serum and adult thymectomy.
Experientia 33 526-528

Waksman, B. & Namba, Y. (1976)
On soluble mediators of immunologic regulation.
Cell. Immunol. 21 161-176

Ward, P. A., Offen, C. D. & Montgomery, J. R. (1971)
Chemoattractants of leukocytes, with special reference to lymphocytes. Fed. Proc. 30 1721-1724

Ward, P. A., Unanue, E. R., Goralnick, S. J. & Schreiner, G. F. (1977)
Chemotaxis of rat lymphocytes.
J. immunol. 119 416-421

Weinbaum, G. & Burger, M. M. (1973)
Two component system for surface guided reassociation of animal cells. Nature (Lond.) 244 510-512

Weisberger, A. S, Guyton, R. A., Heinle, R. W. & Storaasli, J. P. (1951
The role of the lungs in the removal of transfused lymphocytes.
Blood 6 916-925

Wenk, E. J., Orlic, D., Reith, E. J. & Rhodin, J. A. G. (1974)
The ultrastructure of mouse lymph node venules and the passage of lymphocytes across their walls. J. ultrastructure res. 47 214-241.

Wigzell, B. & Andersson, B. (1969)

Cell separation on antigen-coated columns.

J. exp. med. 129 23-36

Wiig, J. N. (1976)

Surface structure influencing the migration in vivo (ecotaxis) of murine thymocytes, T- and B-cells.

Acta pathol. microbiol. Scand. 84C 59-67

Woodruff, J. J. (1974)

Role of lymphocyte surface determinants in lymph node homing.

Cell. Immunol. 13 378-384

Woodruff, J. J. & Gesner, B. M. (1968)

Lymphocytes: circulation altered by trypsin.

Science (N.Y.) 161 176-178

Woodruff, J. J. & Gesner, B. M. (1969)

The effect of neuraminidase on the fate of transfused lymphocytes.

J. exp. med. 129 551-567

Woodruff, J. J., Katz, I. M., Lucas, L. E. & Stamper, H. B. (1977)

An in vitro model of lymphocyte homing. II. Membrane and cytoplasmic events involved in lymphocyte adherence to specialised high-endothelial venules of lymph nodes.

J. immunol. 119 1603-1610

Yakir, Y., Koor, A. I. & Trainin, N. (1978)

Enrichment of in vitro and in vivo immunologic activity of purified fractions of calf thymic hormone. J. exp. med. 148 69-83

Zatz, M. M. (1976a)

Lymphocyte trapping: differential effects on anti-thymocyte serum and irradiation on trapping in lymph nodes and spleen. Immunology 30 749-753

Zatz, M. M. (1976b)

Effects of BCG on lymphocyte trapping

J. immunol. 116 1587-1591

Zatz, M. M., Gingrich, R. & Lance, E. M. (1972a)

The effects of histocompatibility antigens on lymphocyte migration in the mouse. Immunology 23 665-675

Zatz, M. M., Goldstein, A. L., Blumenfeld, O. O. & White, A. (1972b)

Regulation of normal and leukaemic lymphocyte transformation by sodium periodate oxidation and sodium borohydride reduction. Nature New Biology 240 252-255

Zatz, M. M. & Lance, E. M. (1970)

The distribution of chromium-51 labelled lymphoid cells in the mouse. A survey of anatomical compartments. Cell. Immunol. 1 3-17

Zeiller, K., Holzberg, E., Pascher, G. & Hannig, K. (1972)

Free flow electrophoretic separation of T and B lymphocytes - evidence for various subpopulations of B cells. Hoppe-Seyler's Z. physiol. chem. 353 105-110

Zucker, R. M. & Helfman, D. M. (1976)

Thymus subpopulations: density gradients and volume distribution studies. Cell. Immunol. 22 43-52