THE BLOOD AND LYMPHATIC CAPILLARIES OF LYMPH NODES IN THE SHEEP FOETUS AND THEIR INVOLVEMENT IN CELL TRAFFIC

B. Morris, M. Al Salami

Department of Immunology, John Curtin School of Medical Research, Australian National University, Canberra, Australia

While lymphatics and blood vessels have readily identifiable structural differences, they also have different functional affinities for cells of the haemopoietic system and, in regard to these cells, different physiological responsibilities. Blood vessels are concerned with the transport of red cells to the tissues to provide oxygen and to remove carbon dioxide, and with the distribution of leucocytes and platelets throughout the body. Lymphatics, on the other hand, transport predominantly lymphocytes from the tissues, principally the lymph nodes, back to the blood stream. Peripheral lymphatics carry both lymphocytes and macrophages to lymph nodes from the somatic tissues (1,2).

Most lymphocytes present in peripheral and central lymph are recirculating cells moving from the blood into the lymphoid tissues and back to the blood via the lymph (3,4). In the adult animal this traffic is highly ordered being restricted to lymphocytes and denied for the most part to the other blood cells. Further order in the traffic is apparent in that the movement of certain classes of lymphocytes is restricted or promoted through particular tissues, leading to a reassortment of the lymphocyte populations as they migrate between the blood and the lymph (5).

This highly directed and ordered cell traffic is an expression not just of the attributes of the cells involved, but also of the attributes of blood and lymphatic vessels. It depends on particular affinities between the lymphocytes and components of the blood vascular endothelium which promote the egress of certain types of cells from the blood whilst at the same time preventing other cells from escaping. It seems that particular blood vessels in certain tissues have functional properties in relation to the migration of cells which distinguish them from vessels in other tissues. Once outside the blood there is no evidence that lymphatic endothelium plays any selective role in the uptake of cells from the tissues. The lymphatics appear to be readily accessible to all types of migrant cells.

The question remains whether these functional differences are related to morphological differences in the vascular endothelium and its associated cells, to the presence of some recognition structures on the surface of lymphocytes and/or endothelial cells or to the expression of some biotactic influence, derived from the endothelium itself or from associated tissue cells, acting on the lymphocytes. There is also a question as to the ontogeny of this traffic in regard to both the blood and lymphatic vessels and the migrating cells, and whether its exclusiveness for lymphocytes has some special immunological connotation.

Experiments in foetal sheep with chronic indwelling lymphatic fistulae have established, by direct analysis, that the
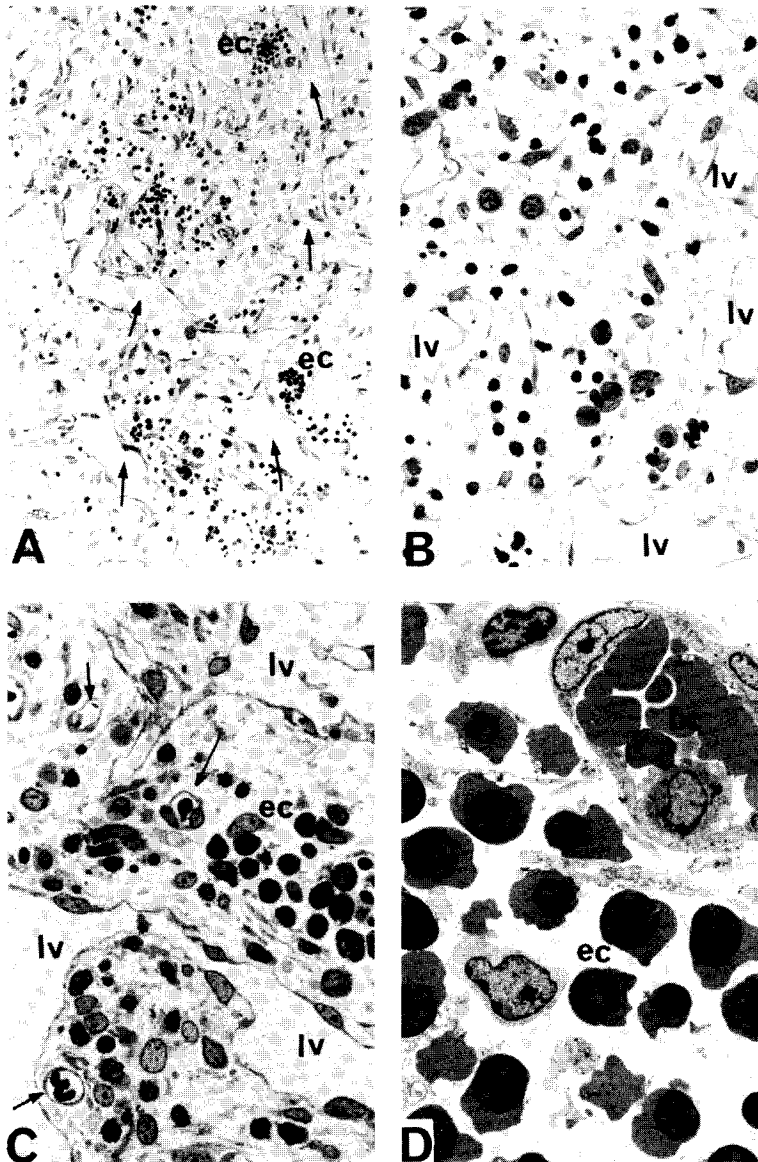
Fig. 1. (A) Popliteal node 60 days gestation. Thin-walled vessels (arrows) containing an occasional cell are present throughout the section. These vessels are probably lymphatics. The clusters of dark staining cells outside the vessels are erythroid colonies (ec). Magnif x150. (B) Popliteal node 60 days gestation. There are several thin-walled empty vessels (lv) in the section which are probably lymphatics. The parenchyma of the node is sparsely populated with mesenchymal cells (some in mitosis), erythroid precursors and a few lymphocytes. Magnif x300. (C) Popliteal node 60 days gestation. Showing empty lymphatics (lv) and several small blood capillaries (arrows) containing red cells. Between the lymphatics is an erythroid colony (ec). Magnif x500. (D) Popliteal node 85 days gestation. Showing a blood capillary (bc) with red cells and a leucocyte. Outside the capillary is a colony of normoblasts (ec). Magnif. x2000.

recirculation of lymphocytes from blood to lymph occurs *in utero*, at least as early as 100 days gestation (6). These experiments have also allowed the numbers of recirculating lymphocytes in the developing foetus to be calculated, and the time taken by the different classes of lymphocytes to complete their recirculation between the blood and the lymph to be measured. The fact that the recirculation of lymphocytes is established in foetal sheep in the absence of circulating immunoglobulins and extrinsic antigen, argues against this activity being immunologically determined. Rather it seems that the metastatic behaviour of lymphocytes is an intrinsic characteristic which, unlike with other cells, is retained throughout the life of the animal.

The development of the blood and lymphatic vasculature of lymph nodes in the sheep foetus

Some further insights into the functional differences of blood and lymphatic vessels in relation to the traffic of cells in the developing foetus are revealed from an examination of the ontogeny of the vasculature of lymph nodes and of the processes of cell migration into foetal lymph nodes. It seems that there are certain shifts in the extent to which cells enter and leave lymph nodes by way of blood vessels and lymphatics at different stages of foetal development (7).

Lymph nodes can first be detected in the sheep foetus around 60 days gestation. The most precocious and most rapidly growing lymph nodes are the prescapular and the mediastinal: nodes such as the hepatic, prefemoral and the mesenteric nodes develop some days later. Initially the lymph nodes are represented by a collection of fine blood vessels surrounded by a thin, ill-defined capsular membrane. It is not possible at this time to distinguish morphologically between blood vessels and lymphatics, even taking into account the cells contained within them. Initially, the blood and lymph vessels are distributed amongst a reticular framework of cells comprising mainly

primitive mesenchymal cells and the occasional lymphocyte, myeloid cell and megakaryocyte *(Fig. 1)*. The vessels are for the most part capillaries and venules whose walls are comprised of a single layer of flat, attenuated, endothelial cells and they resemble closely the structure of authentic lymphatic capillaries. The primordial nodes have no cortical or medullary lymph sinuses at this time nor is there any evidence of structures which will subsequently form the lymph cords, the cortex or the medulla.

Between 60-65 days the various lymph nodes have become connected to the peripheral and central lymphatics of their respective drainage areas and lymphatic capillaries and sinuses within the node can be distinguished from blood vessels *(Fig. 1C)*. The capsule and subcapsular sinus of the node have now become defined together with connections to the afferent lymphatic vessels. The endothelium lining the subcapsular sinus, the lymphatic sinuses and lymph vessels within the node is phagocytic and foreign particles presented to the node in the lymph are taken up into these cells *(Fig. 2B)*. This functional attribute distinguishes the lymphatic vessels from the blood capillaries. Lymph sinuses permeate the node from the subcapsular space to the medulla and the large numbers of lymphatic capillaries are present in the node. The lymphatics and the subcapsular sinus often contain a quite heterogeneous population of cells consisting of macrophages, lymphocytes, megakaryocytes and normoblasts *(Figs. 3 and 4)*. The structure of these vessels is very similar to that of the thin-walled blood capillaries and venules. The heterogeneous cell population within the lymphatic vessels suggests that, at this early stage, the migration of cells between the blood, tissues and lymph is a fairly random process involving most classes of cells.

Once lymphatic connections have been established to and from the nodes cells begin to congregate in the node around the periphery, beneath the subcapsular sinus *(Fig. 4)*. These cells again are a heterogeneous population compris-
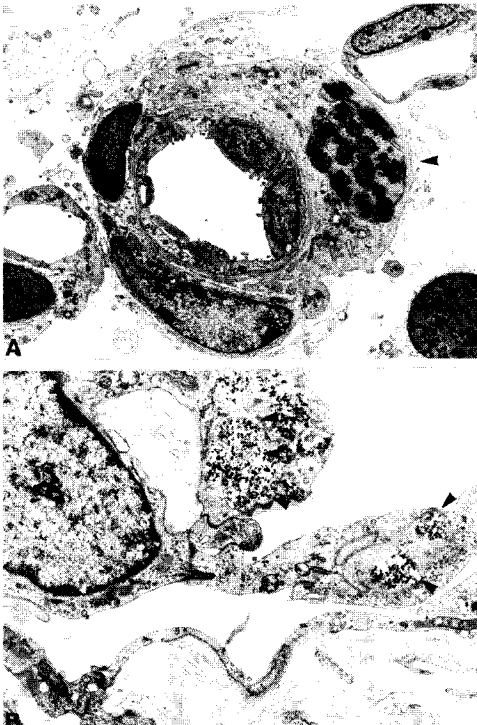
Fig. 2. (A) Blood capillaries in a prescapular lymph node 71 days gestation. The pericyte (arrow) is in mitosis. At this stage of developoment the lymphocyte population in the node is sparse. The cell in the lower right hand corner is a mesenchymal cell. Magnif x4000. (B) Lymphatic endothelium in the popliteal lymph node 70 days gestation. Colloidal carbon had been injected into the hind leg of the foetus 1 hour previously. The carbon particles (arrows) have been taken up into phagocytic vesicles within the cytoplasm of the endothelial cells. Magnif x12,000.

lear detritus from differentiating erythropoietic cells (Fig. 3B, Fig. 4).

In adult sheep the extent of the cell traffic into peripheral lymph nodes such as the prescapular and popliteal nodes by way of the peripheral lymph is less than 10% of the cell traffic leaving the node via the efferent lymph. A significant proportion of the cells are veiled macrophages which do not appear to leave the node (2). While the migration of cells into the node via the peripheral lymph may be of relatively small numerical significance, it is possible that the lymph macrophages may play a role in regulating lymphocyte traffic into and out of the
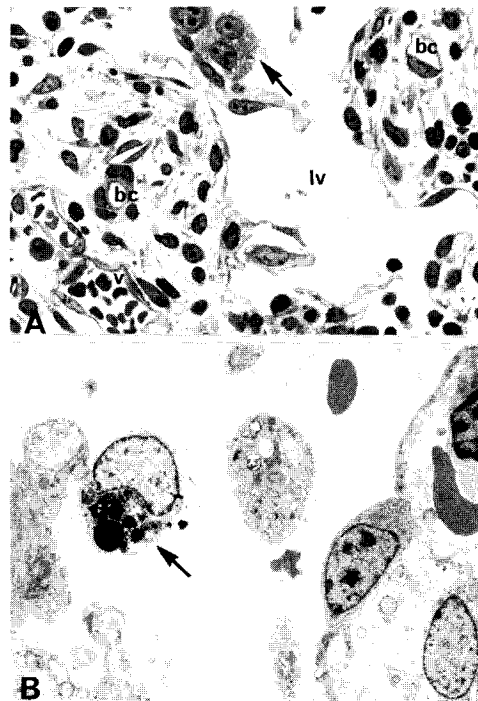

Fig. 3. (A) Popliteal node 75 days gestation. A lymphatic (lv) in the medulla of the node, with 2 megakaryocytes (arrow) within the lumen. The attenuated endothelium of the lymphatic is structurally similar to the endothelium of the adjacent venule (v). There are two small blood capillaries (bc) in the parenchyma one with associated pericytes. Magnif x650. (B) Popliteal node 85 days gestation. The subcapsular sinus contains two macrophages with well developed phagocytic vacuoles. One macrophage (arrow) contains phagocytosed material probably derived from expelled erythroid nuclei. Magnif x3400.

ing small and large lymphocytes, mesenchymal cells with various transitional morphologies and macrophages. In addition, myeloid and erythroid cells and megakaryocytes appear in the nodes. These are found within and adjacent to the subcapsular space and in the lymphatic sinuses (Fig. 4C and D). The distribution of the blood capillaries in the node is most dense in the developing cortical area whereas the lymphatic capillaries are found more frequently in the medulla. In some foetuses, the subcapsular sinus of peripheral nodes contains large numbers of cells, principally macrophages, which are often filled with nuc-
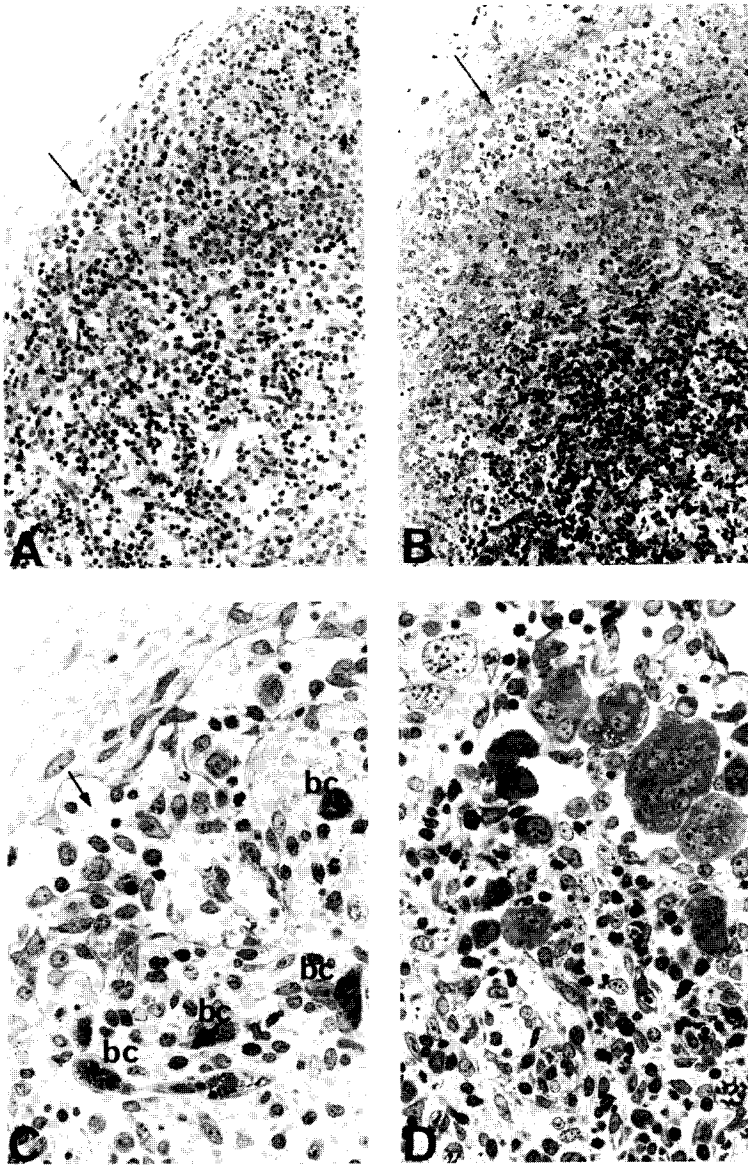
Fig. 4. Cell popoulations in the subcapsular sinuses of lymph nodes in the sheep foetus. (A) Popliteal node 70 days gestation. Cells in the subcapsular sinus arriving in the node via the peripheral lymph. Magnif x250. (B) Prescapular node 81 days gestation. Dilated subcapsular sinus filled with cells. The area towards the medulla is populated with dark staining erythroid cells. Magnif x250. (C) Prescapular node 70 days gestation showing many macrophage cells in the subcapsular sinus (arrow). There are several blood capillaries (bc) filled with red cells in the tissue adjacent to the inner endothelial lining of the sinus but at this stage there are few lymphocytes in the node. Magnif x500. (D) Prescapular node 81 days gestation. There is a cluster of some 10 megakaryocytes in the subcapsular sinus of the node; some have penetrated into the adjacent cortex. Magnif x500.
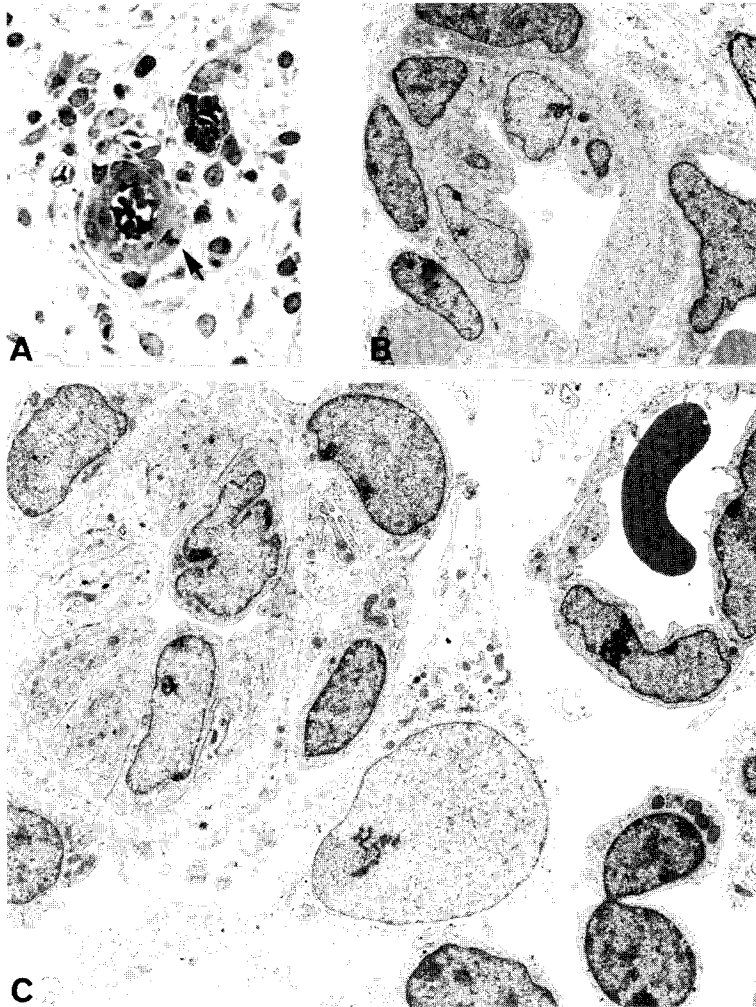
Fig. 5. (A) Light microscope picture of a high endothelial venule in the popliteal node, 70 days gestation. There is a thin walled venule and two small capillaries adjacent to the HEV. The arrow indicates a pericyte in mitosis. There are very few lymphocytes in the tissue. Magnif x800. (B) A low power electronmicroscope picture of a high endothelial venule in the prescapular node, 71 days gestation. There are several pericytes associated with the vessel but no lymphocytes. The nuclear chromatin of the endothelium is finely dispersed and condensed into a very thin margin. Magnif x3000. (C) High endothelial venule and blood capillary in a popliteal lymph node 68 days gestation. Magnif x12,500.

node. Removal of the afferent lymph supply to a lymph node causes a rapid reduction in the cell content of the node (8). No direct measurements have been made on the number of cells in peripheral lymph of early foetuses but there are macrophages and lymphocytes present in peripheral lymph of sheep foetuses from at least 120 days gestation (Simpson-Morgan, personal communication). Histological evidence suggests that the peripheral lymph is likely to be a relatively more important route for the entry of cells into lymph nodes in the foetus than it is in the adult *(Fig. 4)*.

At around 75-80 days gestation, the

primary distinction in the node between the cortex and medulla has become apparent in terms of the relative densities of the cell populations in these two regions *(Fig. 4B)*. Lymphoid cells have begun to segregate into cords bordered by a phagocytic reticuloendothelium which outlines the lymphatic sinuses *(Fig. 2B, Fig. 3A)*.

The lymph nodes of adult sheep are supplied for the most part by blood capillaries and venules that are lined with conventional flattened endothelial cells. The high endothelial venules that are such an obvious feature of the lymphoid tissues of rodents and other species are scarce and there is no evidence in sheep that the migration of lymphocytes from the blood is focused on vessels with high endothelium. Migrating cells can be readily found passing through venules with endothelium that is quite flat. In the lymph nodes of the sheep foetus venules with typical high endothelium are present from the outset. The endothelial cells of these vessels have diffuse granular nuclear chromatin and are often associated with proliferating pericytes *(Fig. 2A, Fig. 5A)*. The high endothelial vessels do not have any special congregations of lymphoid cells either within their walls or in the adjacent tissue and, in fact, many of them are present in locations within the open parenchyma of the node where there are very few cells *(Fig. 5)*. It thus appears that these particular blood vessels are present in the lymph nodes of the foetus before there is any established circulation of lymphocytes from the blood stream to the node. As a consequence the proposition that high endothelium is a functional modification of blood vessels that occurs in association with lymphocyte traffic seems not to be correct. It seems also that there is nothing about the morphology or physiology of high endothelial cells, at least at this stage in the ontogeny of lymph nodes, that specifies the directed migration of lymphocytes from the blood into the node.

A further feature of the blood and lymphatic vasculature of the lymph nodes of the sheep foetus is the presence of haemopoietic stem cells within both classes of vessels throughout most of *in utero* life. Whilst there is an increasing accumulation of lymphoid cells in the nodes and in the lymphatics from 70 days on, there is also erythropoietic, myelopoietic and megakaryopoietic activity in the nodes throughout foetal life *(Fig. 1C and D, Fig. 4B)*. Haemopoiesis is more evident in the cortex of the nodes but nearer to term, haemopoiesis may be occurring in any part of the node. Lymphopoiesis occurs principally in the cortex in structures that could be equated with primary lymphoid follicles but even near to term, the extent of ^{3}H thymidine incorporation into lymphoid cells in foetal nodes is quite limited compared with the thymus and the Peyer's patch follicles (9).

Whilst this description of haemopoiesis in foetal lymph nodes applies generally, nodes in different locations have different degrees of involvement in the various aspects of haemopoiesis. For example, mediastinal, lumbar and hepatic nodes usually exhibit a higher level of erythropoiesis than do the popliteal, prefemoral or prescapular nodes. One form of haemopoiesis may also be very obvious in one lymph node and essentially absent in another. Because of this, the involvement of lymphatic vessels in the transport of these cells into and out of the various nodes differs in different parts of the lymphoid apparatus.

The point to be made out of this involvement of foetal lymph nodes in haemopoiesis is the apparent generalized migration of cells into lymph nodes during the early stages of their development. This suggests that there is no distinction in terms of special migration sites between the various haemopoietic elements and the blood vessels in the developing nodes. While the final level of representation of any particular class of cell in the node will be determined by a variety of events other than migration, it seems that the exquisitely precise discrimination between lymphocytes and other blood cells that is a feature of the cell traffic into mature lymph nodes is

not established in foetal nodes until later in their development. The functional characteristics of blood vessels which specify the egress of lymphocytes from the blood are thus not present from the outset but are acquired during ontogeny. The acquisition of this discriminatory capability may relate to changes in either recognitive mechanisms associated with the developing vascular endothelium, the developing lymphocyte population or the establishment of a population of cells within the node whose activities regulate lymphocyte migration.

REFERENCES

1. Morris, B: Migration intratissulaire des lymphocytes du mouton. Nouvelle Rev.francaise d'Haematologie 8 (1968), 525-534.
2. Smith, JB, GH McIntosh, B Morris: The traffic of cells through tissues: A study of peripheral lymph in sheep. J. Anat. (Lond.) 1 (1970), 87-100.
3. Gowans, JL: The recirculation of lymphocytes from blood to lymph in the rat. J. Physiol. Lond. 146 (1959), 54-69.
4. Hall, JG, B Morris: The origin of the cells in the efferent lymph from a single lymph node. J. Exp. Med. 121 (1965), 901-910.
5. Fahy, VA, B Morris, W Trevella, CF Zukoski: The physical and functional heterogeneity of circulating lymphocyte populations. Blood Cells 6 (1980), 11-19.
6. Pearson, LD, MW Simpson-Morgan, B Morris: Lymphopoiesis and lymphocyte recirculation in the sheet fetus. J. Exp. Med. 143 (1976), 167-186.
7. Al Salami, M, MW Simpson-Morgan, B Morris: Haemopoiesis and the development of immunological reactivity in the sheet foetus. In *Immunology of the Sheep*, Morris & Miyasaka (Eds.), Editiones Roches, Basle, Switzerland, (1985), 19-36.
8. Drayson, MT, WL Ford: Afferent lymph and lymph borne cells: Their influence on lymph node function. Immunobiol. 168 (1984), 362-379.
9. Morris, B: The ontogeny and comportment of lymphoid cells in fetal and neonatal sheep. Immunological Reviews 91 (1986), 219-233.

Bede Morris, B.V.Sc., D.Ph.D.
Head of Immunology
John Curtin School of Medical Research
Australian National University
GPO Box 334
Canberra, A.C.T. 2601
AUSTRALIA