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Lymphocyte Circulation in the Spleen

MARGINAL ZONE BRIDGING CHANNELS AND THEIR POSSIBLE ROLE IN CELL TRAFFIC

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Summary. Histological evidence is presented for distinct, anatomically determined pathways in the spleen for cells in transit between the white pulp and the red pulp prior to entering the draining veins. In rats and mice these appear as narrow channels of lymphocytes which run between both the periarteriolar lymphatic sheath and the red pulp sinuses, and the peripheral white pulp and the red pulp sinuses, crossing the marginal zone in association with fine argentophilic fibres. These marginal zone bridging channels were found to contain labelled T or B cells 4 and 8 hours after injection which suggested that transit was occurring in the direction from white pulp to red pulp rather than the reverse.

Additional histological evidence is given to suggest that, after antigenic stimulation, germinal centre dissociation occurs by release of the germinal centre cells towards the periarteriolar lymphatic sheath before they are shed into the red pulp through marginal zone bridges occurring in the periarteriolar region.

The data are incorporated into a scheme of unidirectional lymphoid cell flow through the spleen. This proposes that the spleen is composed of many functionally discrete units in which the anatomical matrix, reflected by the reticulin fibre pattern, plays a major role. It further implies that the periarteriolar region of the spleen is not totally thymus dependent.

INTRODUCTION

In the course of studies on the migration of labelled lymphocytes through the spleen (Mitchell, 1972) it became apparent that little information existed about the anatomical route by which lymphocytes leave the spleen white pulp to enter the venous circulation.

It is well established that lymphocytes enter the spleen in the arterial circulation which terminates as penicillary arterioles mainly in the marginal zone after traversing the white pulp (Weiss, 1964). The elegant studies of Ford and Gowans (1967) and Ford (1969) involving perfusion of the isolated rat spleen have established that large numbers of labelled lymphocytes which enter the spleen by the arterial route eventually leave *via* the draining veins.

A problem which seems to have been largely ignored is by what route lymphocytes from the white pulp may reach the red pulp sinuses. The establishment of a specific exit route would add to our knowledge of the anatomy of the spleen and would assist in interpretation

of quantitative studies of the sequential location of labelled lymphocytes of different origin within the spleen.

Another related anatomical problem which has received little recent attention is the route by which the products of germinal centre growth are released from the white pulp.

These two questions on the functional anatomy of the spleen are considered in the light of current knowledge about the circulation of lymphocytes of the thymus derived (T cell) and bone marrow-derived (B cell) lineages.

EXPERIMENTAL METHODS

The data consist of histological and radioautographic evidence accumulated during the examination of both rat spleens (after the injection of carbon or ^{125}I-labelled antigen) and mouse spleens (after the injection of ^{3}H-uridine-labelled lymphocytes or ^{125}I-labelled antigen). The experimental methods are described fully in three other papers (Mitchell & Abbot, 1971; Mitchell, 1972; Mitchell, Pye, Holmes & Nossal, 1972). Briefly (a) normal adult rats were injected intravenously with carbon (India ink diluted $\frac{1}{2}$) and their spleens removed 4 minutes later; (b) adult rats which had been drained of their thoracic duct lymph for 5 days were injected intra-arterially into the spleen with ^{125}I-labelled polymerized flagellin from *Salmonella adelaide* (POL) and their spleens removed 4 minutes later; (c) adult CBA mice, which had been injected intraperitoneally with 0·5 ml 20% sheep erythrocytes 6 days previously, were injected intravenously with 5×10^6 labelled lymphoid cells, either (i) thymus-derived (T) spleen cells from adult irradiated, thymus reconstituted CBA mice, (ii) bone-marrow-derived (B) spleen cells from adult thymectomized, irradiated, bone marrow reconstituted CBA mice, or (iii) thymus cells from normal adult CBA mice; the donor cells were labelled by *in vitro* incubation with 20 μCi/ml ^{3}H-uridine for 1 hour; the recipient mice were killed at $\frac{1}{2}$, 4, 8, 24 and 48 hours after injection and their spleens removed; (d) adult mice, littermates of the congenitally athymic ('nude,' nu/nu) mice and themselves either nu/+ or +/+ genotype but of non-nude phenotype, were injected into all footpads with a total of 25 μg ^{125}I-labelled POL. They were killed at 1 hour, 1, 5 or 10 days later and their spleens removed.

In all cases the splenic tissue was fixed in formalin, embedded in paraffin and, where relevant, processed for radioautography using Eastman-Kodak NTB-2 liquid emulsion.

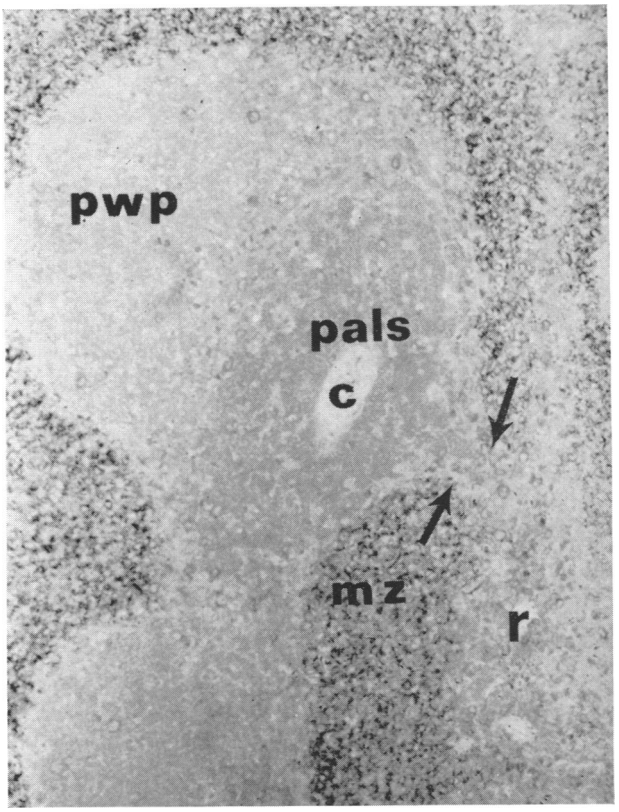
RESULTS

A. RAT SPLEENS

When carbon particles of India ink had been injected intravenously or intra-arterially into rats (Mitchell and Abbot, 1971) they caused a visible demarcation of the marginal zone in animals killed up to 4 minutes after injection. This labelling, which was due to the largely intercellular deposition of carbon, allowed a definition of the normally indistinct border between marginal zone and red pulp. Figs. 1 and 2 demonstrate the typical appearance of a white pulp nodule which is separated from the red pulp by an envelope of carbon-labelled marginal zone. In several places on one longitudinal section of rat spleen, the white pulp could be seen to have channels which penetrated the marginal zone envelope and entered the red pulp directly.

A noticeable feature of these bridging channels was the alignment of lymphocytes

running at an angle to the plane of the sectioned central arteriole. The lymphocyte channels were usually narrow, about 5–10 cells in diameter (approximately 25 μm). Examination of serial sections frequently showed the channel to be apparent in the same location over only 3–6 serial 5-μm sections, indicating a roughly cylindrical three dimensional plan. The alignment of the cells suggested that some form of physical boundary



FIGS 1–3. Sections of spleens from rats killed 4 minutes after the intravenous injections of carbon (diluted India Ink). The carbon is localized in the marginal zone and provides a convenient demarcation of this zone from the white pulp and red pulp. With this marker small breaks in the marginal zone are evident by the absence of carbon.

FIG. 1. Arrows point to a marginal zone bridging channel of lymphocytes, some pyroninophilic, lying between the periarteriolar lymphocyte sheath and the red pulp. (MG-P, Ilford 108+302 filters, × 126.)

existed to retain the lymphocytes (Fig. 3a). Serial sections were therefore stained in series with haematoxylin and eosin (H & E), silver (Sainte-Marie and Sin, personal communication), methyl green pyronin and Masson's stain. It was found that wherever a bridging channel of lymphocytes was demonstrated with H & E or methyl green pyronin, the serial section contained argentophilic fibres aligned along the 'direction' of the lymphocytes of the channel. The fibres of the bridging channel were frequently at a tangent to the fibres of the periarterial lymphatic sheath (Fig 3a, b). Collagen was not demonstrated in the channels with Masson's stain.

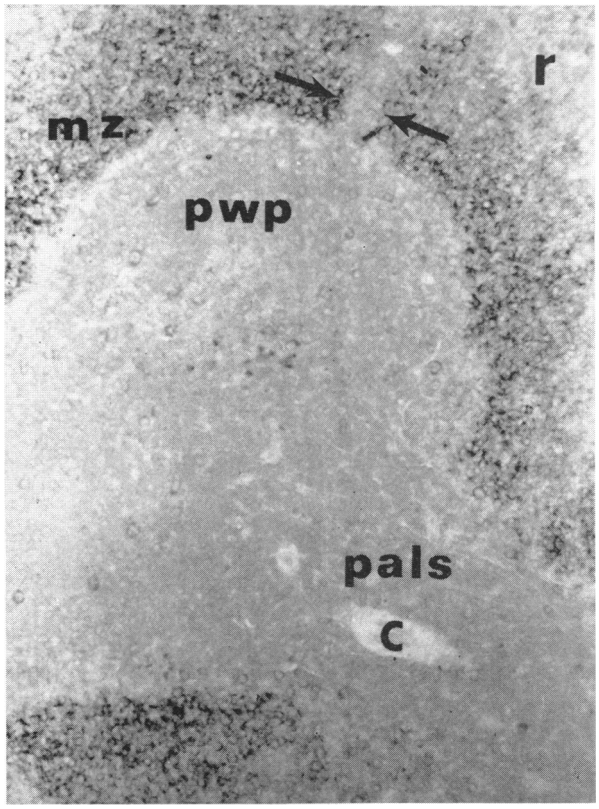


FIG. 2. Arrows point to a marginal zone bridging channel of lymphocytes lying between the peripheral white pulp and the red pulp. (MG-P, Ilford 108+302 filters, × 125.)

B. MOUSE SPLEENS

(i) *Marginal zone bridging channels*

In comparison with sections of rat spleen, mouse spleen showed less well demarcated borders between white pulp and marginal zone as well as between the marginal zone and red pulp. Nevertheless occasional channels of lymphocytes similar to those seen in the rat were apparent as bridges through the marginal zone between the white and the red pulp (Fig. 4a, b). The absence of erythrocytes in the bridge was demonstrated by using a deep violet filter (Ilford 501) to photograph a section stained with methyl-green pyronin.

The possible importance of these bridging channels was suggested by radioautography after the injection of ^{3}H-uridine-labelled cells. When the spleen cells injected had been labelled thymus-derived-(T) spleen cells, labelled lymphocytes were found in the periarteriolar lymphocyte sheath (Fig. 5) and also occasionally in bridging channels. When bone-marrow-derived (B) spleen cells had been injected the B-cells were concentrated in the peripheral white pulp, but occasionally they, too, were found in bridging channels (Fig. 4a, b).

In some cases, favourable sections of the periarteriolar region revealed rounded collections of lymphocytes quite clearly demarcated from the surrounding pyroninophilic

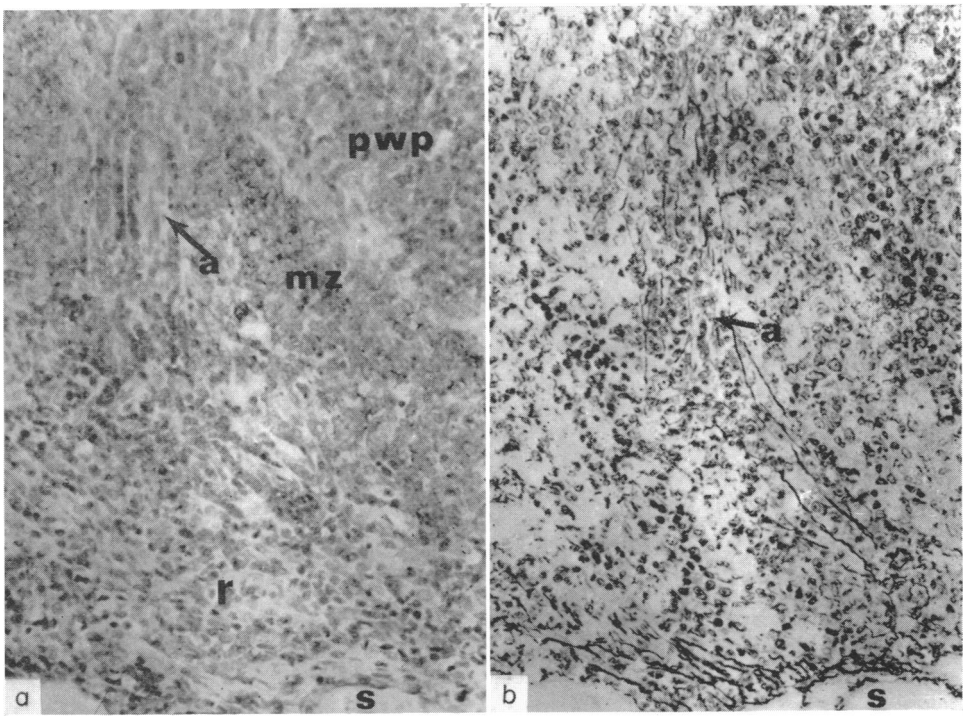


FIG. 3. Serial sections of a marginal zone bridging channel (× 248). (a) MG-P stain. A fine arteriole (a, arrow) is evidently travelling in the same direction as the parallel arrays of lymphocytes traversing the carbon demarcated marginal zone. (b) Silver stain. The fine arteriole (a, arrow) is no longer clear, but the parallel argentophilic fibres have a tenuous connection with the heavier reticulin network of the red pulp sinus. The rest of the marginal zone is characteristically free of reticulin fibres.

periarteriolar cells (Fig. 5). These appeared to be transversely cut periarteriolar channels distended by their content of lymphocytes. Labelled T-cells or thymus cells could be found amongst the closely packed non-pyroninophilic cells in these channels from 4 to 48 hours after intravenous injection, but B cells were not commonly found. The occurrence of true lymphatics has been shown by Snook (1946) in this region in other species. A definite fibre pattern of reticulin exists around the arteriole (Weiss, 1964; Sainte-Marie and Sin, 1968) and it seems probable that pseudolymphatics created by the reticulin fibres could be causing the channelling of lymphocytes in this area in mice and rats. Further suggestive evidence of periarteriolar lymphatics appeared in the white pulp of rats drained of their thoracic duct lymph for 5 days. As Fig. 6 shows, there were vessel-like spaces in the periarteriolar region as though cells had been drained out of lymphatic-like periarteriolar channels.

Despite the many sections examined, only once was there the appearance that a channel of lymphocytes had emanated from the periarteriolar region at a tangent to the direction of the arteriole (Fig. 7a–c). This appeared to be an alignment of lymphocytes bounded by reticulin fibres. It seemed that this was a chance section of an apparently continuous bridge of fibres between the periarteriolar sheath and a marginal zone bridging channel. It was not present in the two adjacent sections.

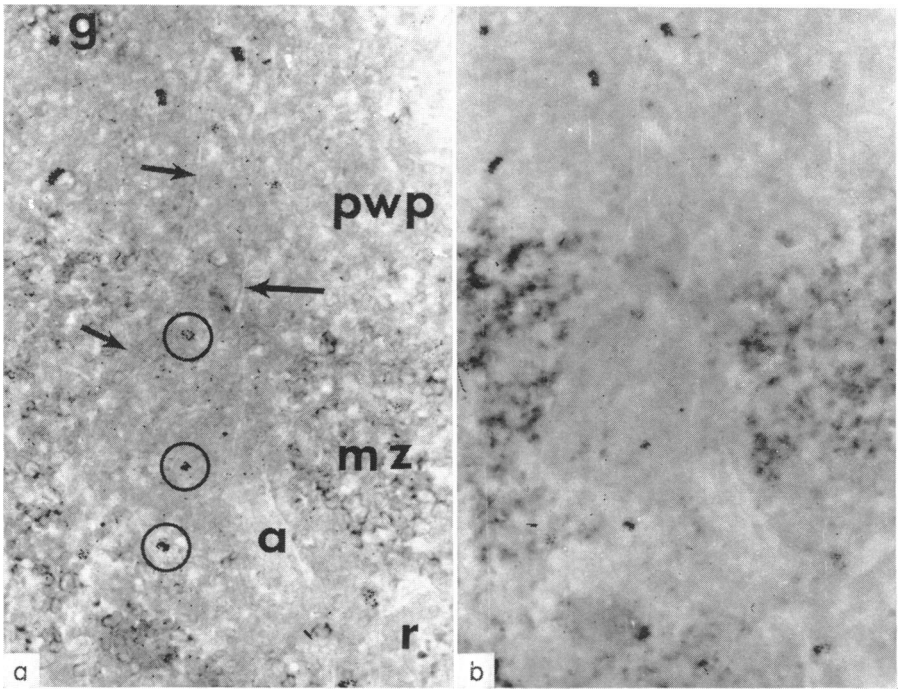


FIG. 4. One section from a mouse injected 8 hours previously with ^{3}H-uridine labelled bone-marrow-derived (B) spleen cells. The B cells were most concentrated in the peripheral white pulp including germinal centres. Some labelled cells (circles) were in the occasional marginal zone bridging channels characterized by unstained reticulin fibres (arrowed) and a fine arteriole. (×208.) Radioautograph exposed 8 weeks. (a) MG-P stain, Ilford 108+302 (green) filters. (b) MG-P stain, Ilford 501 (violet) filter. This filter emphasizes the erythrocytes and thereby marks the marginal zone.

(ii) *Germinal centre dissociation*

Interesting histological features emerged in mice which had been injected intraperitoneally with sheep erythrocytes (0·5 ml 10 per cent suspension) 6 days before the injection of ^{3}H-uridine labelled cells (Mitchell, 1972). Mice killed up to 2 days after the introduction of the labelled cells (up to 8 days after antigen) showed massive germinal centres and prominent periarteriolar collections of pyroninophilic cells. There was a high concentration of large pyroninophilic cells and even macrophages with an appearance similar to that of tingible body macrophages (Fig. 9) in the periarteriolar region. By following serial sections, and occasionally in one section, the periarteriolar pyroninophilic cells gave the appearance of being connected to the arterial pole of a germinal centre (Fig. 8). Visual tracking of the periarteriolar pyroninophilic collections through serial sections revealed occasional sections where the area of pyroninophilia extended from the central arteriole right through the border of the periarteriolar lymphocyte sheath, through the marginal zone and into the red pulp (Fig 7a, b and 9). The appearance of Fig. 9 was somewhat reminiscent of the 'germinal centre dissociation' described by Congdon and Makinodan (1961). However, the streaming of cells through the marginal zone did not emanate from the marginal zone pole of a well-formed germinal centre, but rather from a *periarteriolar* collection of germinal-centre-like cells.

Some histological appearances in the material examined were consistent with germinal

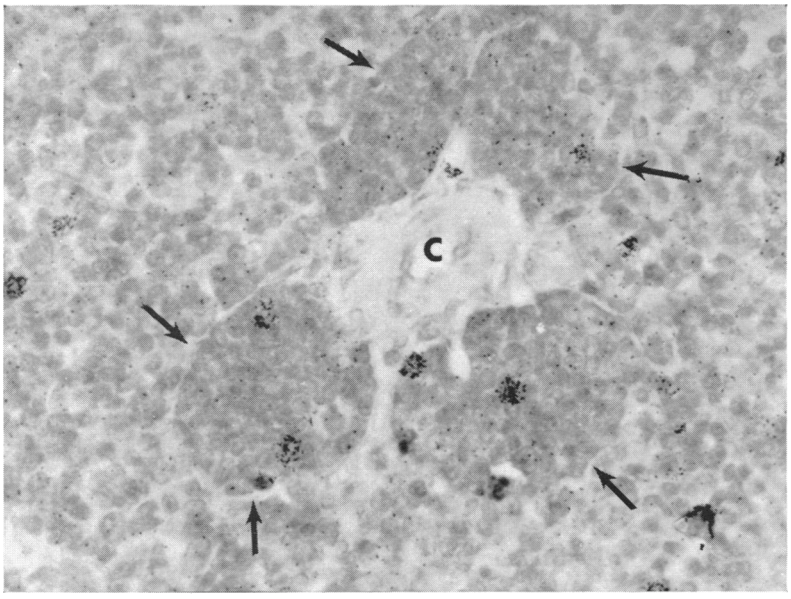


FIG. 5. Spleen of a mouse injected 24 hours previously with ^{3}H-uridine labelled thymus-derived (T) spleen cells. Most cells localized in the periarteriolar lymphocyte sheath. This mouse had been given sheep erythrocytes intraperitoneally 6 days previously and the spleen showed massive germinal centres in the peripheral white pulp, together with considerable pyroninophilia in the periarteriolar region. This increased cellular activity, together with the increased traffic caused by the T cell injection, apparently caused periarteriolar pseudo-lymphatics (arrowed) to become extended. A large proportion of the labelled T cells were found in these channels. (MG-P stain, no filter, ×448.) Radioautograph exposed 6 weeks.

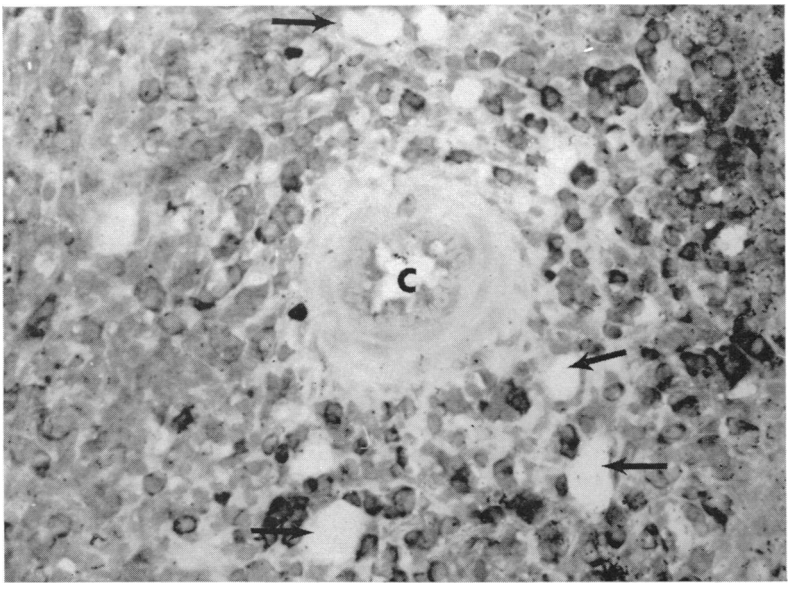


FIG. 6. Spleen of a rat 4 minutes after the intra-arterial injection of ^{125}I-labelled antigen following 5 days of thoracic duct lymph drainage. The radioautographic grains are virtually confined to the marginal zone which borders the depleted white pulp. The central arteriole is surrounded by empty channels (arrowed) which could be followed over serial 5-μm sections. (MG-P stain, Ilford 108+302 (green) filters ×368.) Radioautograph exposed 4 weeks.

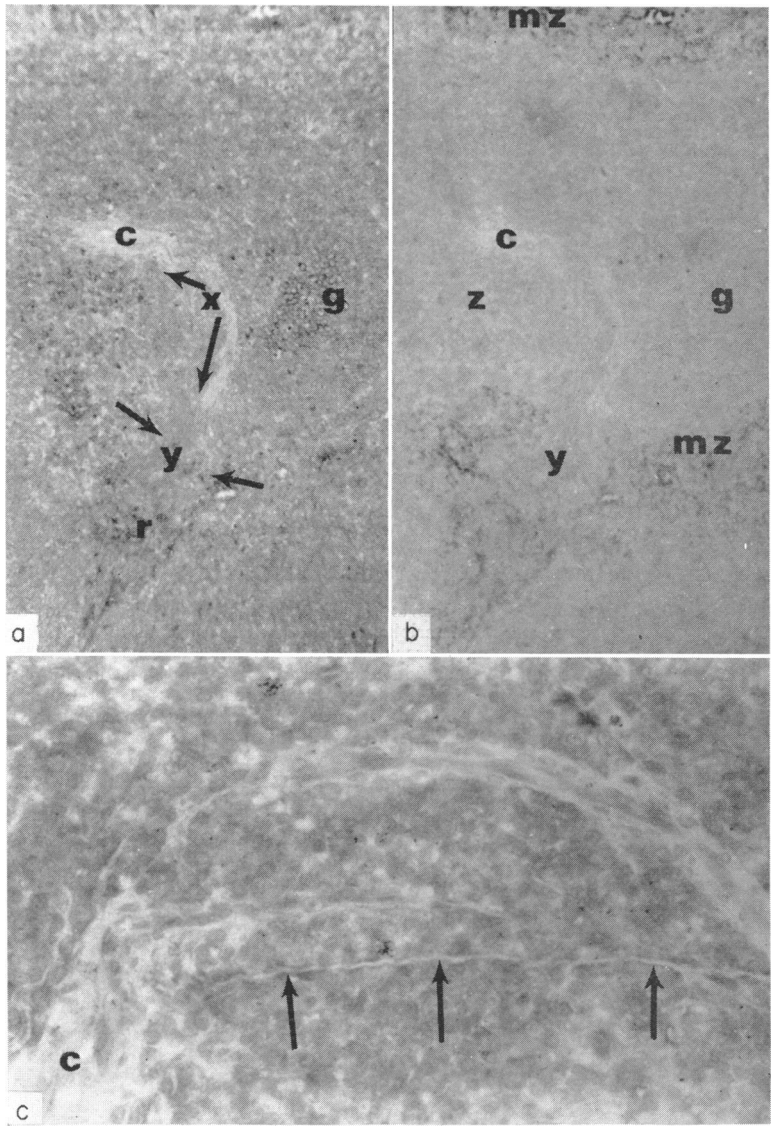


FIG. 7. One section from the spleen of a mouse 8 hours after the injection of ^{3}H-uridine labelled thymus cells and 6 days after intraperitoneal injection of sheep erythrocytes. MG-P stain, radioautograph exposed 6 weeks. (a) (Magn. ×88, Ilford 108+302 (green) filter.) White pulp with a germinal centre, central arteriole with intense periarteriolar pyroninophilia reaching out to the red pulp. The finer arteriole branching out to the periphery becomes associated with a line of unstained fibres (arrow X) seen virtually in continuity from the periarteriolar region to the marginal zone bridging channel (arrow Y). (b) (Magn. ×80, Ilford 501 (violet) filter.) The emphasis on erythrocytes provides a map of the white pulp-marginal zone border. By comparison with (a) this shows that area Y is a marginal zone bridging channel and that area Z suggests release of the periarteriolar pyroninophilic cells to the red pulp. At first glance area Z might appear to be the 'dissociation' of a germinal centre into the marginal zone. (c) (×368, no filters.) Area marked arrow X above to show the relationship of the unstained fibre network (arrowed) to the arteriolar region.

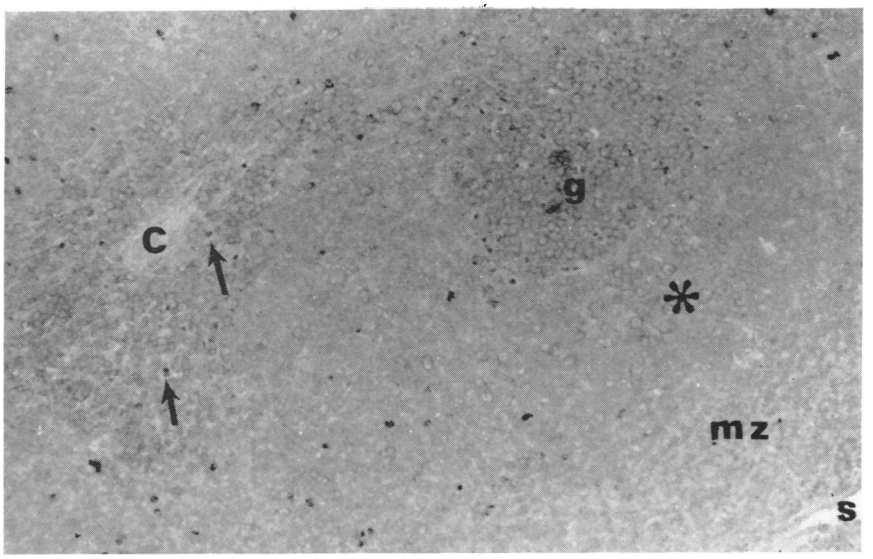


FIG. 8. Spleen of a mouse injected 48 hours previously with ^{3}H-uridine-labelled B spleen cells, and 8 days previously with sheep erythrocytes. These are labelled cells predominantly in the peripheral white pulp but occasionally in the periarteriolar pyroninophilic collections. The massive germinal centre appears to be in continuity with the periarteriolar collection which contains elements like tingible body macrophages (arrowed). There are pyroninophilic cells at the peripheral pole of the germinal centre (asterisk) but these do not extend out into the marginal zone. (MG-P stain, Ilford 108+302 (green) filters, × 128.) Radioautograph exposed 8 weeks.

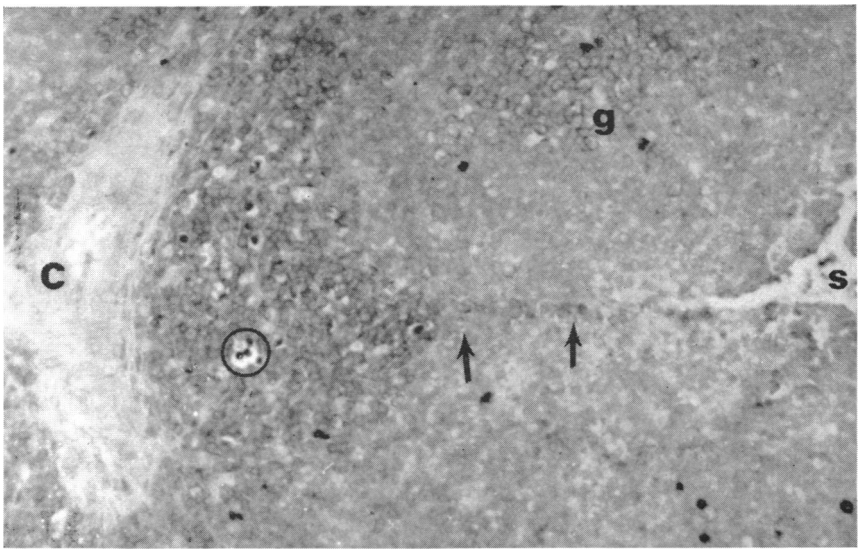


FIG. 9. Spleen from the same mouse as Fig. 8. Higher magnification reveals the alignment of the pyroninophilic periarteriolar collection by unstained fibres and the cells of tingible body macrophage character (circled). At one point (arrowed) the collection appears to be about to burst out into the red pulp sinus region along the channel indicated. (MG-P stain, Ilford 108+302 (green) filters, × 176.)

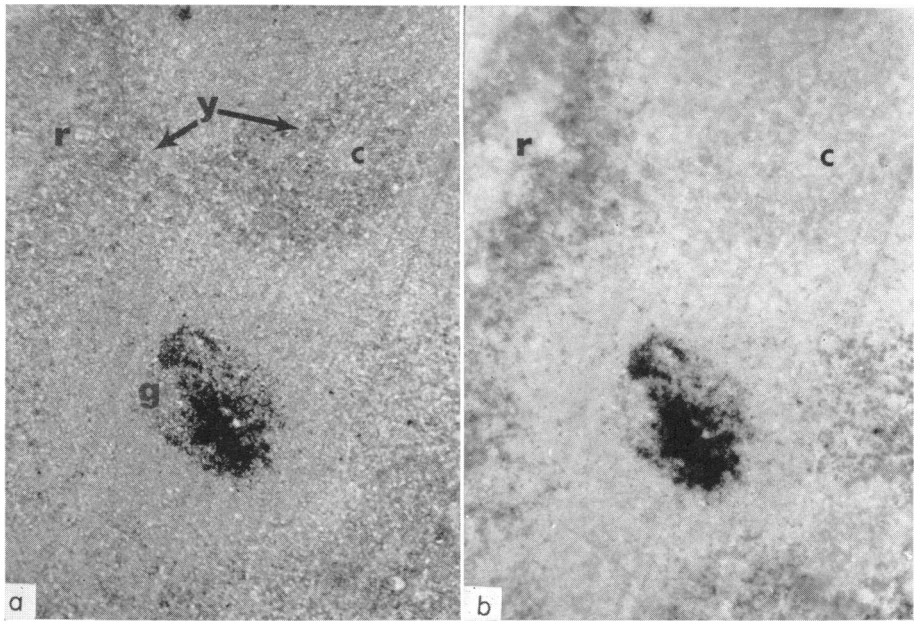


FIG. 10. One section from a mouse injected 10 days previously with ^{125}I-labelled polymerized flagellin from *Salmonella adelaide*. The label is localized on the dendritic follicular web of a well developed germinal centre. Without the label, the area of periarteriolar pyroninophilia (arrow Y) would appear similar to the germinal centre and could give rise to the appearance of a germinal centre disgorging its contents across the marginal zone. Although the periarteriolar collection exhibits pyroninophilia and tingible body macrophages it does not exhibit antigen localization. (MG-P stain, ×88.) Radioautograph exposed 4 weeks. (a) Leitz filter, blue. (b) Ilford filter, 501, violet.

centre products (pyroninophilic blast cells) being released outwards individually from a true secondary follicle into the marginal zone; that is, individual pyroninophilic blast cells were seen between the peripheral pole of the germinal centre and the marginal zone. More often, however, the apearances were consistent with the products being released inwards towards the central arteriole. It seems more likely that the germinal centre 'dissociation' occurred inwards towards the central arteriole to form the periarteriolar pyroninophilia and that the peripheral blast cells were migrants from the blood. Cells destined to leave the white pulp to form pyroninophilic nests in the red pulp may do so by leaving from the periarteriolar region via bridging channels which occur intermittently along the length of the periarteriolar sheath. Where gross hypertrophy of germinal centres had occurred this could give the superficial appearance of a germinal centre disgorging its contents across the marginal zone.

Further evidence that the periarteriolar pyroninophilic collections were not classical germinal centres came from radioautographs of mouse spleen 10 days after the injection of ^{125}I-labelled polymerized flagellin (Mitchell, Pye, Holmes and Nossal, 1972). Labelled antigen was localized on the dendritic follicular network of the germinal centres in the peripheral white pulp (Fig. 10) but was never seen in association with the germinal-centre-like aggregations in the periarteriolar region or with the stream of such cells bridging the periarteriolar region and the red pulp sinus.

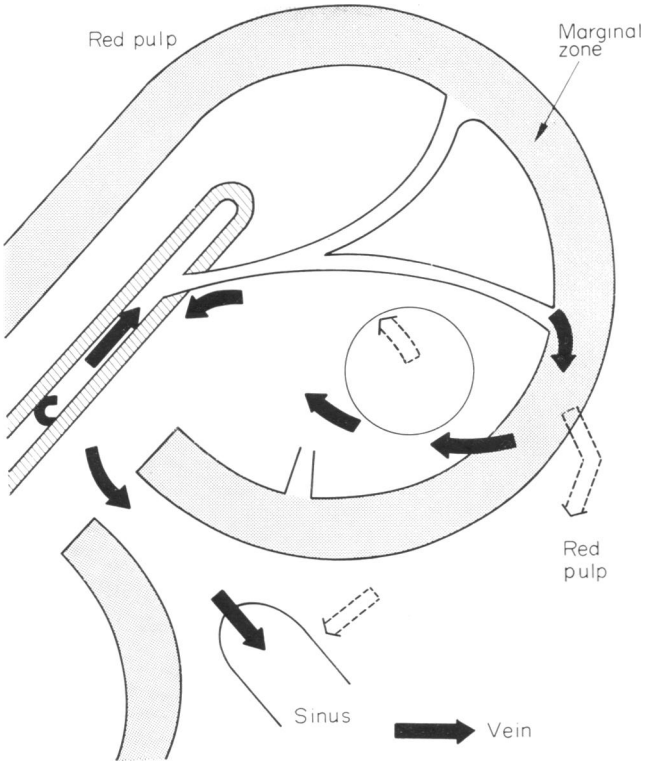


FIG. 11. Possible routes for cell circulation in the spleen. Cells enter with the blood and enter the parenchyma of the spleen in the marginal zone. Some may pass directly into the red pulp, whilst others enter the white pulp. Cells of bone marrow origin may exhibit preferential localization in the follicular regions of the peripheral white pulp, whilst thymus-derived cells pass quickly to the periarteriolar zone. All lymphocytes destined to re-enter the *venous* circulation may do so by passing through the occasional marginal zone bridging channels.

DISCUSSION

It is now well established that lymphocytes enter the spleen via the arteries which traverse the white pulp and terminate as fine arterioles, mainly in the marginal zone (Snook, 1964; Weiss, 1965). Lymphocytes are released from the arterioles mainly in the marginal zone then either pass directly into the red pulp or traverse the marginal sinus and pass into the peripheral regions of the expanded periarteriolar lymphocyte sheath (Pettersen, Borgen and Graupner, 1967; Goldschneider and McGregor, 1968; de Sousa, 1971; Ford, 1969; Mitchell, 1972).

Since the spleen possesses an efferent lymphatic drainage (Snook, 1946) it seems probable that some of the white pulp lymphocytes leave the spleen in this lymph to join the recirculating pool. The method by which this occurs has yet to be demonstrated but probably involves the periarteriolar channels referred to in a WHO Report (1970) 'besides the (central) artery and running parallel to it there appears to be a channel lined by flattened cells which is usually collapsed and empty but may after stimulation be distended with small lymphocytes. It appears to be an exit pathway for cells of the recirculatory pool, not unlike the exit pathway from the paracortex of the lymph node.'

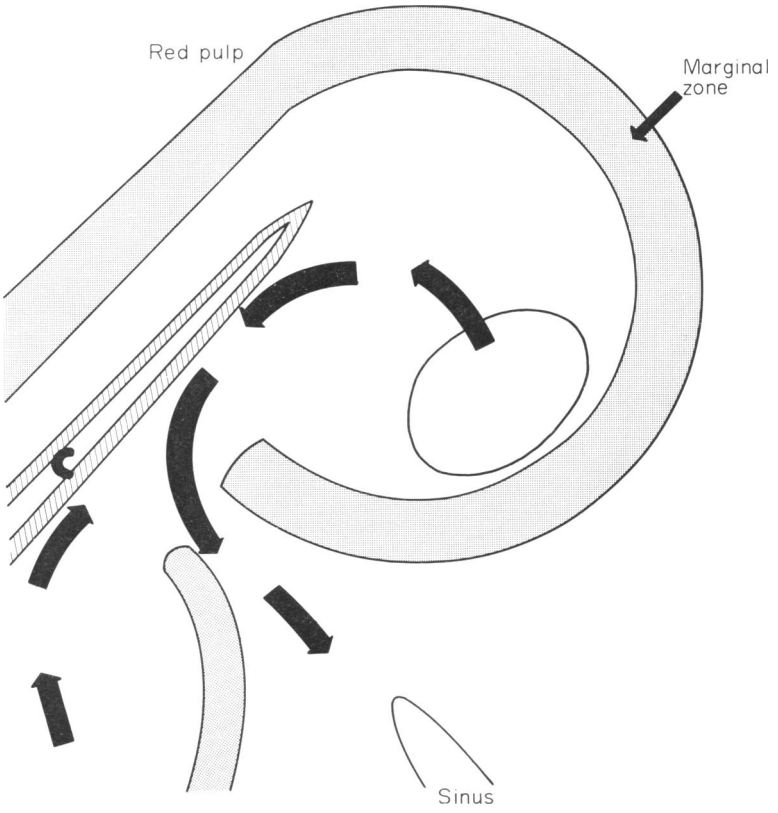


FIG. 12. Possible route for germinal centre dissociation within the spleen. Products of germinal centres migrate to the periarteriolar zone then pass into the red pulp at discrete points where marginal zone bridging channels emanate from the periartersolar reticulin sheath and pass into the red pulp.

True lymphatics have been shown to occur in the periarteriolar region of the spleens in other species (Snook, 1946; Weiss and Janout, 1972). In mice and rats a definite fibre pattern of reticulin exists around the arteriole (Weiss, 1964; Sainte-Marie and Sin, 1968) and it seems probable that lymphatics or pseudolymphatics are created by the reticulin fibres to cause the channelling of lymphocytes in this area.

Although some cells of the white pulp probably leave via the lymph, Ford (1969) established that large numbers of labelled lymphocytes which enter the spleen by the arterial route eventually leave via the draining veins. Many of the labelled, thymus-derived lymphocytes in his studies passed through the white pulp and were found in the periarteriolar regions; they then left the white pulp to re-enter the venous circulation with a transit time of about 4 hours. The mode of exit has not previously been determined (Gowans, 1966; Parrott and De Sousa, 1971).

Three possible routes may be envisaged for lymphocytes passing from the white pulp into the venous blood. First, they may circulate in the white pulp and then migrate back to the periphery, through the marginal sinus into the marginal zone and thence into the red pulp cords and sinuses. No good evidence has been found to either support or refute this possibility but it has often been tacitly assumed (Clark and Weiss, 1971).

Secondly, it could be postulated that lymphocytes in the white pulp re-enter the

arterioles and then circulate to the red pulp via the marginal zone. This postulate should perhaps be considered analogous with lymphocyte passage through the post-capillary venules in the lymph node cortex (Gowans and Knight, 1964). Certainly, labelled thymus derived cells are found clustered around the arterioles several hours after injection (Parrott, De Sousa and East, 1966; Ford, 1969). Furthermore, these arterioles have been described as 'leaky structures' (WHO, 1970). No studies, however, demonstrated lymphocytes in transit through the arteriolar walls and this possibility seems to be remote.

The third possibility is that specific exit channels for lymphocytes exist between the white and the red pulp. Passing reference to such exit points, but without histological data have been made by Congdon and Makinodan (1961) and in the report of a WHO Scientific Group (WHO 1970) which states that 'there are connexions between the white pulp and the peripheral tissue, but only at separate points'.

The histological evidence presented here suggests the existence of bridging channels between the white and red pulp in the spleens of mice and rats. The network of reticulin fibres associated with the channels of lymphocytes often seems to be directly connected with a red pulp sinus. The existence of anatomically determined channels would allow a biologically attractive hypothesis for a route of cell circulation within the spleen. This is presented in Fig. 11. An undirectional flow of cells is proposed which seems to be consistent with the data presently available. All cells entering the white pulp do so from the marginal zone. Thymus-derived cells then pass quickly through the peripheral white pulp to the periarteriolar lymphocyte sheath (Parrott, De Sousa and East, 1966; Goldschneider and McGregor, 1968; Ford, 1969). Bone-marrow-derived cells also enter the peripheral white pulp (De Sousa, 1971; Mitchell, 1972; Sprent, 1972). They tend to reach a steady concentration in the peripheral regions which could indicate either (a) that they pass more slowly through the periphery than T cells; or (b) a certain proportion of them localize in this region whilst others pass on to the periarteriolar region.

For those cells which are destined to leave the spleen white pulp, two routes of exit could be taken, namely via the efferent lymph or via the blood. Experimental evidence is required to show whether labelled cells leave via periarteriolar pseudolymphatics which drain into the true trabecular lymphatics (Snook, 1946). Those cells which leave via the blood, however, must pass from the white pulp to the draining veins of the red pulp. It is proposed that this involves passage through the anatomically distinct channels described.

Furthermore, the histological data presented suggests that the products of cell proliferation in germinal centres may follow a similar direction of flow to lymphocytes within the spleen. This is represented diagramatically in Fig. 12. Two essential differences exist between this proposed mechanism of germinal centre dissociation and that previously described (Congdon and Goodman, 1962; Hanna, 1964; Fliedner, 1964). First, Hanna proposed (1964) that germinoblast exit was at the peripheral pole of the germinal centre (that is on the marginal zone side in the spleen). This mechanism appeared to be supported by evidence based on quantitative grain counts of ^{3}H-thymidine uptake in germinal centres of both spleen (Hanna, 1964) and tonsillar crypts (Koburg, 1967). Other circumstantial evidence was the appearance of pyroninophilic cells between the marginal zone of the spleen and the germinal centres (Pettersen, Borgen and Graupner, 1967). However, as these authors noted, the direction of movement of these cells could not be determined by a series of static pictures. Furthermore the interpretation of ^{3}H-thymidine uptake depends on the assumption (noted by Hanna) that no cell traffic occurs through germinal centres.

Currently available data shows that the integrity of the germinal centre (Parrott, 1967) is far from complete. The localization of bursal-derived cells in the germinal centres in chickens (Durkin, Thies & Thorbecke, 1970) and of bone-marrow-derived spleen cells in germinal centres (Mitchell, 1972) invalidates this concept and imposes limitations on the interpretation of the previous grain count data. Further circumstantial evidence against an analogous germinal centre dissociation in lymph nodes occurring via the peripheral cap comes from the kinetic studies of Olson (1971) and from the fact that tracts of pyroninophilic cells are seen in continuity from the deep aspects of germinal centres to the medullary cords (Nossal and Ada, 1971).

A second difference between the two proposed paths of germinal centre dissociation is in the proposition that anatomically defined areas exist for passage of germinal centre products from the white pulp to the red pulp, rather than a release of these cells at random. The presence of streams or compact clusters of pyroninophilic cells in the red pulp favours the idea of localized concentrated entry of precursors from the white pulp. Many of the plasma cell nests in the red pulp are possibly IgM-producing cells whose initial development occurred in the periarteriolar lymphatic sheath (Kim and Watson, 1971; Mitchell *et al.*, 1972). One problem with histological assessments is that germinal centre dissociation does not occur in normal animals in the known absence of an IgM response. However, similar histological studies on the release of preformed germinal centre products in irradiated animals may show periarteriolar pyroninophilia in the absence of a new IgM response by other cells in that region.

Further functional studies on cell circulation in the spleen will be required to correlate the anatomical structure of the arterial system with its sites of termination. It is not known at present where these fine arterioles often associated with the marginal zone bridging channels terminate, since they do not appear to open into the marginal zone. It is possible that these arterioles form a virtually closed circulation to the venous sinuses in contrast to the majority of open ending arterioles which terminate in the marginal zone (Clark and Weiss, 1971). The hypothesis presented reduces the number of possible directions of travel for cells in the spleen and simplifies the concept of cellular dynamics in the spleen marginal zone. It implies that the direction of cell migration after entry into the marginal zone depends on the surface composition of the mobile cell, perhaps determined by its interaction with the permanent reticulum of the anatomical micro-environment. The reticulin network has also been implicated by de Sousa (1971) in determining the route and rate of traffic in the splenic white pulp.

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REFERENCES

CLARK, J. M. and WEISS, L. (1971). 'Effects of a bacterial vaccine on the marginal zone of the spleen.' *Amer. J. Anat.*, **132**, 79.

CONGDON, C. C. and GOODMAN, J. W. (1962). 'Changes in lymphatic tissues during foreign tissue transplantation.' *Proc. Int. Symp. Tissue Transpl.* (Ed. by A. P. Cristoffanini and G. Hoeker). Uni. of Chile, Santiago, Chile.

CONGDON, C. C. and MAKINODAN, T. (1961). 'Splenic white pulp alteration after antigen injection: relation to time of serum antibody production.' *Amer. J. Path.*, **39**, 697.

DURKIN, H. G., THIESS, G. A. and THORBECKE, G. J. (1970). 'Homing of cells from the bursa of Fabricius to germinal centres in the chicken spleen.' *Advanc. exp. Med. Biol.*, **12**. 119.

FLIEDNER, T. M. (1964). 'On the origin of tingible bodies in germinal centres.' *Germinal Centres in Immune Responses*. (Ed. H. Cottier, N. Odartchenko, R. Schindler and C. C. Congdon). p. 218. Springer-Verlag N.Y. Inc.

FORD, W. L. (1969). 'The kinetics of lymphocyte recirculation within the rat spleen.' *Cell. Tiss. Kinet.*, **2**, 171.

FORD, W. L. and GOWANS, J. L. (1967). 'The role of lymphocytes in antibody formation. II. The influence of lymphocyte migration on the initiation of antibody formation in the isolated, perfused spleen.' *Proc. roy. Soc. B.*, **168**, 24.

GOLDSCHNEIDER, I. and MCGREGOR, D. D. (1968). 'Migration of lymphocytes and thymocytes in the rat.' *J. exp. Med.*, **127**, 155.

GOWANS, J. L. (1966). 'Lifespan, recirculation and transformation of lymphocytes.' *Int. Rev. exp. Path.*, **5**, 1.

GOWANS, J. L. and KNIGHT, E. J. (1964). 'The route of recirculation of lymphocytes in the rat.' *Proc. roy. Soc. B.*, **159**, 257.

HANNA, M. G. (1964). 'An autoradiographic study of the germinal center in spleen white pulp during early intervals of the immune response.' *Lab. Invest.* **13**, 95.

JANOUT, V. and WEISS, L. (1972). 'Deep splenic lymphatics, in the marmot: an electron microscope study.' *Anat. Rec.* **172**, 197.

KIM, Y. B. and WATSON, D. W. (1971). 'Histological changes of lymphoid tissues in relation to the ontogeny of the immune response in germfree piglets.' *Advanc. exp. Med. Biol.* **12**, 169.

KOBURG, E. (1967). 'Cell production and cell migration in the tonsil.' In *Germinal Centres in Immune Responses*. (Ed. H. Cottier, N. Odartchenko, R. Schindler, C. C. Congdon). p. 176. Springer-Verlag N.Y. Inc.

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.

MITCHELL, J. and ABBOT, A. (1971). 'Antigens in Immunity. XVI. A light and electron microscope study of antigen localization in the rat spleen.' *Immunology*, **21**, 207.

MITCHELL, J., PYE, J., HOLMES, M. C. and NOSSAL, G. J. V. (1972). 'Antigens in Immunity. XVIII. Antigen localization in "nude" mice.' *Aust. J. exp. Biol. Med. Sci.*, (in press).

NOSSAL, G. J. V. and ADA, G. L. (1971). *Antigen, Lymphoid Cells and the Immune Response*. p. 74. Academic Press, N.Y. and Lond.

OLSON, I. A. (1971). 'The kinetics of germinal centre formation in lymph nodes responding to keyhole limpet haemocyanin.' *Exp. med. Path.*, **14**, 139.

PARROT, D. M. V. (1967). 'The integrity of the germinal centre: an investigation of the differential localization of labelled cells in lymphoid organs.' *Germinal Centres in Immune Responses*. (Ed. H. Cottier, N. Odartchenko, R. Schindler and C. C. Congdon). p. 168. Springer-Verlag, N.Y. Inc.

PARROTT, D. M. V. and DE SOUSA, M. A. B. (1971). 'Thymus-dependent and thymus-independent populations: origin, migratory patterns and lifespan.' *Clin. exp. Immunol.*, **8**, 663.

PARROTT, D. M. V., DE SOUSA, M. A. B. and EAST, J. (1966). 'Thymus dependent areas in the lymphoid organs of neonatally thymectomized mice.' *J. exp. Med.* **123**, 191.

PETTERSEN, J. C., BORGEN, D. F. and GRAUPNER, K. C. (1967). 'A morphological and histochemical study of the primary and secondary immune responses in the rat spleen.' *Amer. J. Anat.*, **121**, 305.

SAINTE-MARIE, G. and SIN, Y. M. (1968). 'Reticular fibre pattern in rat spleen.' *Rev. Canad. Biol.*, **27**, 273.

SNOOK, T. (1946). 'Deep lymphatics of the spleen.' *Anat. Rec.*, **94**, 43.

SNOOK, T. (1964). 'Studies on the perifollicular region of the rat's spleen.' *Anat. Rec.*, **148**, 149.

DE SOUSA, M. (1971). 'Kinetics of the distribution of thymus and marrow cells in the peripheral lymphoid organs of the mouse: ecotaxis.' *Clin. exp. Immunol.*, **9**, 371.

SPRENT, J. (1972). 'Studies on T and B cells in the thoracic duct lymph of the mouse. I. Migratory properties.' *Cell Immunol* (in press).

WEISS, L. (1964). 'The white pulp of the spleen. The relationships of arterial vessels, reticulum and free cells in the periarterial lymphatic sheath.' *Bull. Johns Hopk. Hosp.*, **115**, 99.

WEISS, L. (1965). 'The structure of the normal spleen.' *Seminars in Haematol.*, **2**, 205.

W. H. O. (1970). 'Factors regulating the immune response.' *Wld Hlth Org. techn. Rep. Ser.*, **448**, 28.

ABBREVIATIONS

Used throughout the Figures are

a	fine arteriole in marginal zone bridging channel
c	central arteriole
g	germinal central
m.z.	marginal zone
p.a.l.s.	periarteriolar lymphocyte sheath
p.w.p.	peripheral white pulp
r	red pulp
s	sinus in the red pulp
t	trabecular fibres
MG-P	methyl green pyronin stain.