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THE DIFFERENT MIGRATORY CHARACTERISTICS OF LYMPHOCYTE POPULATIONS FROM A WHOLE SPLEEN TRANSPLANT*

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SUMMARY

Spleens from AS × BN donor rats labelled *in vivo* by multiple doses of [^{3}H]thymidine were transplanted into syngeneic recipients by anastomosis to the abdominal great vessels. The recipients were killed 1–5 days after receiving the whole spleen transplants and the numbers and location of the [^{3}H]thymidine-labelled cells which had migrated from the labelled donor spleen traced by means of autoradiographs of sections, imprints and smears of various recipient lymphoid tissues. These results were compared with the migration pattern of labelled dissociated spleen cell suspensions injected intravenously. The latter consists almost entirely of small lymphocytes which migrate to T or B areas of recipient spleen, lymph nodes and Peyer's patches. The labelled whole spleens also contained cells which migrated to the T and B areas of recipient lymphoid tissues, but in addition contained many lymphoid cells which migrated to the red pulp of the recipient spleen and to the lamina propria of the gut. These experiments showed, therefore, that the spleen contains mobile elements which have not been detected by transfer of spleen cell suspensions.

INTRODUCTION

Previous studies (Parrott & Sousa, 1971; Sousa, Ferguson and Parrott, 1973) on the fate of *in vitro* labelled spleen cells have shown that the majority of labelled cells after i.v. infusion were found in either B or T areas of the recipient's lymphoid tissues, spleen, lymph nodes and Peyer's patches. A reasonable deduction from the earlier studies, from observations on the *in vitro* perfusion of whole spleens (Ford, 1969a) and thoracic duct drainage (McGregor & Gowans, 1963) was that apart from B and T lymphocytes the population of

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the spleen is normally sessile and cannot migrate if introduced into the circulation. Other explanations are that in preparation of a spleen cell suspension most of the red pulp is discarded or that only small lymphocytes which are easily expressed or withdrawn in suspension survive the various *in vitro* manipulations during labelling procedures. Certainly most cells in a single cell suspension of spleen after *in vitro* labelling are morphologically identified as small lymphocytes, and it is significant that the only situation in which some labelled cells were found in the red pulp (Parrott & Sousa, 1971) occurred in experiments in which spleen donors were labelled *in vivo* with multiple injections of [^{3}H]thymidine and the cell suspensions were not submitted to the multiple trauma of *in vitro* labellings and washings. We have now adopted a different approach which we hope will solve some of the problems of the mobility of spleen cell populations. Whole spleens from donors labelled with [^{3}H]thymidine were transplanted into syngeneic recipients by direct anastomosis of the arterial and venous supplies and the migrating labelled spleen cells traced in the recipient by means of autoradiography. By this procedure the normal architecture of the whole spleen was preserved and the constituent cells transferred within their physiological environment were free to migrate out or not at their own tempo. The present experiments did give further insight into the mobility of all the various spleen cell populations especially those normally found in the red pulp. They also revealed a cell component which homed to the lamina propria of the gut, a component which was not detected in spleen cell suspensions (Parrott & Ferguson, 1974).

MATERIALS AND METHODS

Inbred rats of either sex from closed colonies maintained in the Departments of Bacteriology and Immunology or Surgery, Glasgow Western Infirmary, were used throughout this study. Swiss Albino by Brown Norway F1 hybrids (AS × BN) of 200–300 g were used as whole spleen donors and recipients, while histoincompatible August rats (Aug) were used as skin donors.

Splenic transplantation

Isografts of the whole spleen were transplanted and anastomosed to the abdominal great vessels of the recipient by a technique modified from that of Pettirossi (1971).

Preparation of donor spleen

The upper abdominal aorta and coeliac axis of the heparinized donor were isolated, and the hepatic, and right and left gastric arteries ligated. The splenic pedicle was separated from omentum and pancreas, and the junction of the portal and splenic veins cleared of surrounding tissues. Care was taken to ligate all small veins entering the pancreas from the splenic pedicle to obviate venous ooze and haematoma formation upon restoration of the circulation. A segment of aorta at the coeliac axis was removed, following ligation of its distal portion. An elipse of portal vein surrounding the splenic vein was cut, and the spleen and its vascular pedicle removed.

Transfer to recipient

The aortic segment was sutured end-to-side to the infrarenal aorta of the recipient with 8–0 monofilament suture (Ethicon Ltd, Edinburgh), and the portal vein anastomosed in

similar fashion as a patch graft to the inferior vena cava. Ischaemic time was 30–40 min. Arterial pulsations appearing in the coeliac axis and splenic artery immediately upon release of the vascular clamps, gradually spread to vessels in the splenic hilum. Veins filled with blood and the flaccid and cyanotic spleen slowly regained its normal tone and colour. The vascularized graft was placed along the right perinephric and psoas gutter of the recipient, where it remained firm, compact and barely palpable through the flank. On the infrequent occasions when the organ enlarged obviously and became tender, this was found to be a consequence of vascular accident or torsion of the pedicle. All animals were treated similarly post-transplant, receiving food and water *ad libitum* with terramycin (9 gm/l) (Pfizer Ltd, Sandwich) added to their drinking water. The viability of each spleen graft was checked when the recipient was killed.

Time sequence of labelling and transplantation

It was considered inadvisable to use [^{3}H]adenosine or [^{3}H]uridine which, although labelling all the cells in the donor, would present difficulties in removing excess isotope from a whole organ. Accordingly the donor spleens were labelled *in vivo* with multiple doses of [^{3}H]thymidine and the number of labelled cells increased by stimulating cell division in the donors with a skin allograft. [^{3}H]Thymidine (Radiochemical Centre, Amersham) (specific activity 52 Ci/mmol) injection (0·5 μCi/g of body wt) was injected intraperitoneally twice daily from 4 days until 10 days after skin grafting. This time schedule was adopted because previous experience (Tilney & Ford, 1974) had shown that the maximum uptake of label in the spleen of rats rejecting skin grafts occurred during this time.

The labelled spleen was transplanted into untreated syngeneic recipients 1–4 days after cessation of isotope injection. At various time intervals after transplantation (0–48 hr) the thoracic duct was cannulated so as to determine the proportion of labelled cells in the donor spleen capable of recirculating in the thoracic duct lymph. The total volume and number of cells in the collected lymph was counted and cytocentrifuge preparations made.

The spleen graft recipients were killed 1–5 days after transplant and blood smears made, and the following tissues removed: transplanted spleen; recipient's own spleen; liver; lungs; mesenteric, cervical and axillary lymph nodes; the thymus; Peyer's patches; and several segments of gut.

Impression smears were made of the transplanted spleen, the recipient's own spleen and mesenteric lymph nodes. These were fixed in methyl alcohol for 3 min and then processed for autoradiography together with blood and lymph smears. On average, 2000 cells were counted in each impression smear and the number and type of labelled cells determined in autoradiographs.

Autoradiography

All tissue samples were fixed in formol saline, embedded in paraffin wax and sectioned at 3 μm. The mounted sections and smears were coated by dipping them in photographic emulsion (Ilford K5) exposed at 4° for 5–17½ weeks and then developed, fixed and stained with Methyl Green–Pyronin.

Labelled cell counts

In order to quantitate the distribution of the labelled cells over different compartments

of the spleens, lymph nodes and Peyer's patches we scanned and counted several fields (five to ten) of each area using a $50 \times 50\,\mu$m graticule under a $\times 95$ immersion oil lens and a $\times 10$ eye piece. This method of counting has been described previously (Parrott, Ferguson & Sousa, 1973; Parrott & Ferguson, 1974). The different compartments in donor and recipient spleen were red pulp (RP), perifollicular area (PFA), periphery of the Malpighian body (PF) and thymus-dependent area (TDA). All these compartments are clearly differentiated from one another in the rat and are much easier to identify than in the mouse (Parrott & Sousa, 1971). The different compartments of lymph nodes were nodular areas (NA), excluding germinal centres, and thymus-dependent areas (TDA) and medulla. In Peyer's patches the compartments were nodular (B) areas, i.e. the primary nodules and cuff of lymphocytes surrounding germinal centre, and thymus-dependent (T) area, i.e. in the immediate subepithelial and inter-nodular zones. The delineation of the compartments of the lymphoid tissues in B and T areas is known from work on the selective depletion of T areas in thymus-dependent animals (Parrott, Sousa & East, 1966) the selective migration of enriched populations of labelled B or T lymphocytes to specific areas (Parrott & Sousa, 1971; Howard, Hunt & Gowans, 1972; Sprent, 1973; Sousa *et al.*, 1973) and by means of immunofluorescence studies using lymphocyte antisera directed against the cell surface membranes of T or B lymphocytes (Gutman & Weissman, 1972; Goldschneider & McGregor, 1973).

Percentage distribution of labelled cells

In order to compare the results presented here with already published studies on the distribution of dissociated labelled spleen cells, several sections of recipient spleens were selected at random and the total number of labelled cells counted under a $\times 40$ flat field objective. The results were expressed as the percentage distribution of the total number of labelled cells per section within each compartment.

The distribution of dissociated labelled spleen cells

Four (AS $\times$ BN) F1 rats responding to a secondary challenge with BSA injected 7 days previously were killed, their spleens removed, a spleen suspension prepared and labelled with [^{3}H]adenosine *in vitro* by established techniques (Parrott & Sousa, 1971). The labelled spleen cells (8×10^7) were injected intravenously into syngeneic recipients who were killed after 24 hr and autoradiographs of various lymphoid tissues prepared as above.

RESULTS

Eight out of nine spleens transplanted from primed donors into unprimed syngeneic recipients were successful as assessed by post-mortem and histological examination. All tissues in the recipients, including the donor spleen, were normal.

Proportion of labelled cells in a primed donor

Labelled cells were found in large numbers in all areas of the spleen of a normal rat killed immediately after the [^{3}H]thymidine labelling regime (Table 1, Fig. 1). The labelled cells appeared to be of all types: nests of plasma cells and undifferentiated and blast cells in the red pulp; small, medium and large lymphocytes and blast cells in the white pulp.

TABLE 1. Number of labelled cells* per unit area of donor spleen

Rat serial number	Post-isotope days	Post-grafting days	Red pulp (mean ± s.d.)	Perifollicular area (mean ± s.d.)	Periphery of follicle (mean ± s.d.)	Thymus-dependent area (mean ± s.d.)
L2	3	1	39·3 ± 15·4	23·0 ± 6·6	22·0 ± 3·5	10·5 ± 1·7
L9	3	1	23·0 ± 6·7	21·3 ± 4·1	29·0 ± 3·6	20·3 ± 3·5
L6	6	3	38·0 ± 12·6	27·0 ± 4·4	29·7 ± 5·9	18·0 ± 4·4
L7	6	3	20·3 ± 3·5	17·0 ± 7·2	18·0 ± 4·0	18·3 ± 5·1
L1	5	4	6·1 ± 1·9	14·8 ± 2·0	11·0 ± 0·8	10·5 ± 1·7
L4	8	4	9·5 ± 3·4	19·2 ± 4·2	11·7 ± 4·3	13·4 ± 1·9
L5	6	5	17·8 ± 3·2	23·0 ± 8·9	23·3 ± 5·8	19·0 ± 12·7
Ungrafted donor spleen	0	0	52·0 ± 15·1	45·0 ± 16·6	42·00 ± 6·9	41·33 ± 7·6

* Autoradiographs were exposed for 10½ weeks.

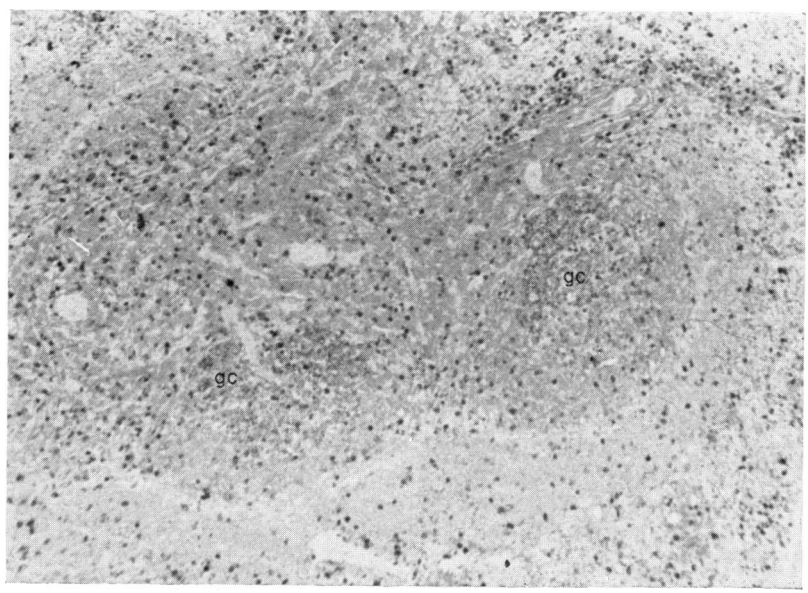

FIG. 1. Section of spleen from primed AS × BN rat killed immediately after [^{3}H]thymidine labelling regime. Note large numbers of labelled cells in all areas of spleen including germinal centres (gc). The autoradiograph was exposed for 10½ weeks. (Methyl Green–Pyronin, magnification × 105.)

Proportion of labelled cells in donor spleens

Sections of autoradiographs of transplanted spleens removed from recipients killed 1 day after operation (and 3 days after cessation of isotope injection) had substantially lower numbers of labelled cells in all areas when compared with non-transplanted primed donor

spleen (Table 1). Thereafter, the rate of decline of cell numbers in spleens removed from recipients at increasing time after grafting was much slower, except possibly in the red pulp. There were detailed changes; in the red pulp of donor spleen from the two recipients killed 1 day after transplant nests of labelled plasma cells, large undifferentiated cells and blast cells were noted. At later times far fewer nests of labelled plasma cells occurred although the large labelled cells persisted. In the white pulp 1 day after transplant, labelled blast cells as well as lymphocytes were still present, but later the blast cells had disappeared. There was a progressive diminution in size and labelling activity of germinal centres. At early times there were a few heavily labelled cells (20–30 grains per cell) with all the rest moderately labelled (5–10 grains per cell) (Fig. 2). The proportion of heavy to lightly labelled cells persisted but with rapid dilution of isotope because of cell division. From 3 days onward, the

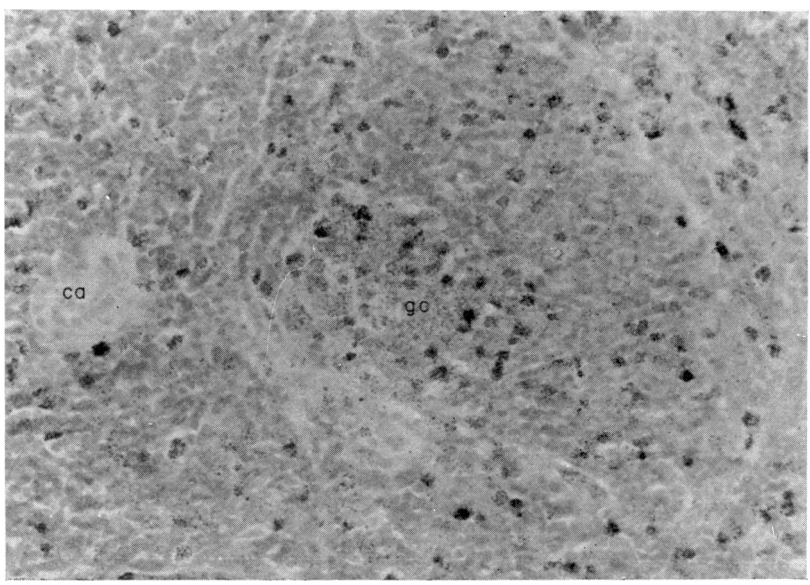

FIG. 2. Section of donor spleen taken from recipient (L2) killed 1 day after transplant. Note the labelled cells in the perifollicular area, periphery of follicle (B area) and thymus-dependent (T) area around the central arteriole (ca). There are some heavily and some moderately labelled cells in the germinal centre (gc). The exposure time was 17½ weeks. (Methyl Green–Pyronin, magnification ×262.)

lightly labelled cells had become too lightly labelled to be considered individually as labelled cells, although it was obvious from the light 'peppering' of the autoradiographs that they had indeed been labelled.

The variety of labelled cells in the transplanted donor spleen was reflected in impression smears made from its cut surface before fixation; with occasional labelled plasma cells and blast cells as well as labelled lymphocytes of all sizes (Table 2).

The distribution of labelled cells in recipient spleens

Labelled cells were already present in the recipient's own spleen 1 day after transplantation, and they were seen in all areas: red pulp; perifollicular area; periphery of the follicle;

TABLE 2. Number of labelled cells* in imprints, blood and thoracic duct lymph smears

Rat serial number	Post-isotope	Post-grafting	Percentage labelled lymphocytes in blood smears	Percentage labelled lymphocytes in thoracic duct lymph†	Impression smears: Donor spleen (Percentage labelled lymphocytes)	Impression smears: Recipient spleen (Percentage labelled lymphocytes)	Impression smears: Recipient mesenteric lymph node (Percentage labelled lymphocytes)
L2	3	1	2·1	n.d.	n.d.	n.d.	n.d.
L9	3	1	5·8	3·3	9·6	1·4	0·5
L6	6	3	6·9	n.d.	6·1	2·5	0·3
L7	6	3	7·8	1·9	8·8	2·8	0·5
L1	5	4	3·9	n.d.	n.d.	n.d.	n.d.
L4	8	4	3·2	n.d.	n.d.	n.d.	n.d.
L5	6	5	7·5	3·5	4·3	1·9	0·9

n.d. = Not determined.

* Autoradiograph of impression smears were exposed for 5 weeks, blood smears for 13 weeks and thoracic duct lymph smears for 10 weeks.

† From samples of thoracic duct lymph collected in the 24 hr before death.

TABLE 3. Number of labelled cells* per unit area of recipient spleen

Rat serial number	Post-isotope days	Post-grafting days	Red pulp (mean ± s.d.)	Perifollicular area (mean ± s.d.)	Periphery of follicle (mean ± s.d.)	Thymus-dependent area (mean ± s.d.)
L2	3	1	3·4 ± 1·1	3·0 ± 2·6	5·5 ± 1·3	4·7 ± 2·1
L9	3	1	2·5 ± 1·4	1·7 ± 1·2	8·0 ± 2·8	3·7 ± 2·1
L6	6	3	6·6 ± 1·4	10·0 ± 4·4	8·7 ± 3·1	8·0 ± 2·6
L7	6	3	6·6 ± 3·6	11·8 ± 1·5	7·8 ± 2·2	4·8 ± 1·3
L1	5	4	2·9 ± 0·7	4·0 ± 1·9	5·0 ± 2·3	3·0 ± 1·6
L4	8	4	2·7 ± 1·7	4·3 ± 1·7	4·0 ± 2·2	3·8 ± 1·7
L5	6	5	7·2 ± 1·5	8·7 ± 2·3	7·7 ± 4·2	4·7 ± 1·5

* Autoradiographs were exposed for 10½ weeks.

thymus-dependent area (Fig. 4) (Table 3). Little subsequent change occurred during the period of experiments, i.e. 1–5 days after grafting either in the total number or relative distribution of labelled cells. Unlike the donor spleen no nests of labelled plasma cells were seen in the red pulp at any time, although there were fair numbers of large labelled lymphoid cells. At 1 day after grafting labelled blast cells as well as lymphocytes occurred in the white pulp, but thereafter only small, medium and large labelled lymphocytes were noted. In germinal centres there were one or two fairly heavily labelled cells (Fig. 3), but at no time were all the other cells covered by light grains as in the donor spleen. Only labelled lympho-

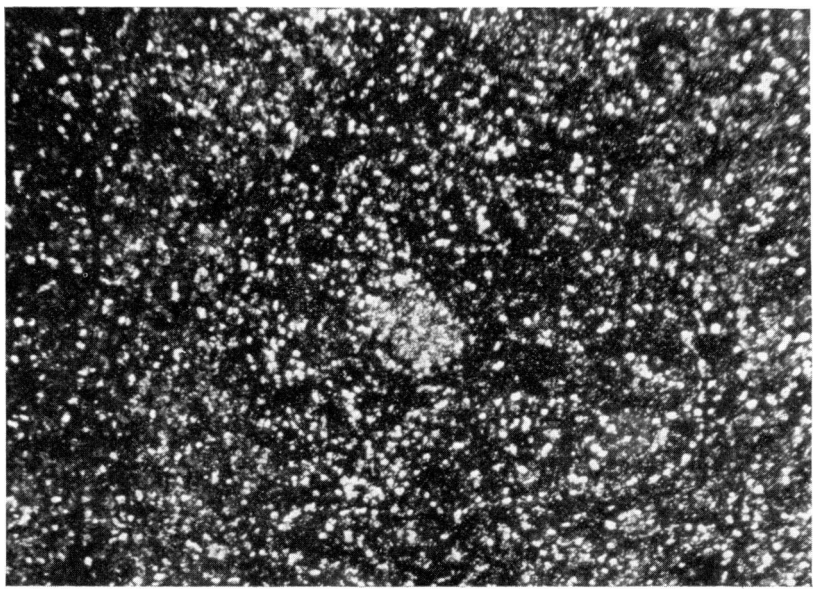

FIG. 3. Section of donor spleen taken from recipient (L2) 1 day after transplant. Note large numbers of labelled cells in all areas including one germinal centre. The exposure time was 17½ weeks. Compare with Fig. 4. Both photomicrographs taken with dark ground condenser. (Methyl Green–Pyronin, magnification × 105.)

cytes were seen in impression smears from the recipient spleen; there were no labelled blasts or plasma cells (Table 2).

Throughout the period of the experiment there were more labelled cells in the donor than recipient spleen (Figs 4 and 5), and the difference between the number of labelled cells in each compartment of donor and recipient spleens was highly significant ($P<0{\cdot}001$). There were indications from both unit area counts and impression smears that the difference

TABLE 4. Percentage distribution of [^{3}H]thymidine-labelled cells* in recipient spleen

Rat serial number	Post-isotope days	Post-grafting days	Red pulp	Perifollicular area	Periphery of follicle	Thymus-dependent area
L2	3	1	28·4	24·1	25·9	21·6
L9	3	1	27·4	25·8	28·9	18·4
L6	6	3	48·1	14·1	23·8	14·1
L7	6	3	26·2	34·8	28·9	10·2
L1	5	4	35·2	13·1	18·2	18·4
L4	8	4	50·05	16·6	24·3	8·8
L5	6	5	42·2	23·0	17·8	17·6

* Autoradiographs were exposed for 10½ weeks.

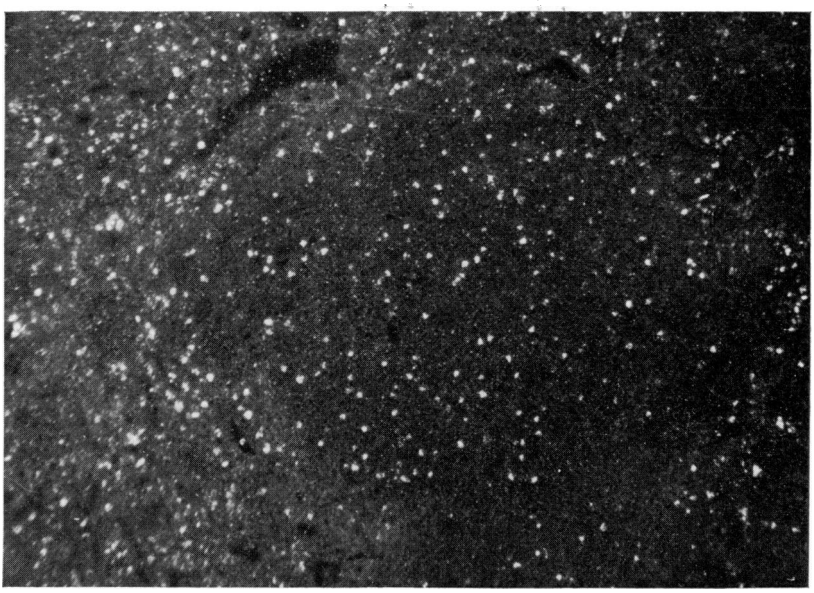

FIG. 4. Section of recipient spleen taken from recipient (L2) 1 day after transplant. Note the difference in numbers of labelled cells when compared with donor spleen similarly spread over all areas but excluding germinal centres. The exposure time was 17½ weeks. (Methyl Green–Pyronin, magnification × 105.)

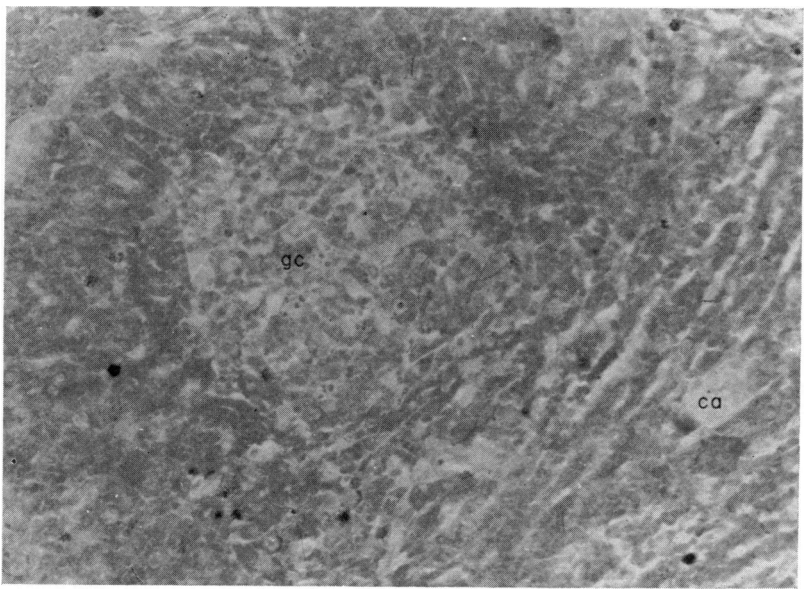

FIG. 5. Recipient spleen (L2) 1 day after transplant. Cells in both T and B areas but fewer than in donor spleen (compare with Fig. 2) and only one or two in germinal centres (gc). Central arteriole (ca). The exposure time was 17½ weeks. (Methyl Green–Pyronin, magnification × 262.)

between the two spleens was greatest at 1 day after grafting and then decreased, but this was by no means consistent. A possible complication could have arisen if a higher rate of cell division and dilution of label occurred in the recipient than donor spleen, although a comparison of autoradiographs exposed for different lengths of time did not indicate that this was a major source of error.

[^3H]Adenosine-labelled dissociated spleen cells injected intravenously

It is obvious from the unit area counts of recipient spleens that there were similar numbers of labelled cells over all four areas (Table 3). This fact is emphasized in the percentage distribution counts made in complete sections (Table 4). In this respect these results differ from previous findings on the percentage distribution of i.v. injected dissociated spleen cells whether labelled [^{3}H]thymidine, [^{3}H]adenosine or [^{3}H]uridine in the mouse in which after an initial 4 hr after injection the majority of labelled cells were found in the periphery of the follicle and TDA, but not in red pulp or perifollicular area (Parrott & Sousa, 1971; Sousa *et al.*, 1973). In order to reassure ourselves that this was not a species difference, four normal rats were injected with 8×10^7 [^{3}H]adenosine-labelled primed spleen cells. The inoculum contained 94% small and medium lymphocytes, 4·5% large lymphocytes and plasma blasts and 1·5% myeloid cells. Percentage distribution and unit area counts of labelled cells were made on spleen autoradiographs. At 24 hr after injection the cells had assumed the pattern familiar from mouse studies, i.e. the vast majority of cells were within the Malpighian body (Table 5). It is interesting to note that unlike [^{3}H]adenosine-labelled thoracic duct cells (Howard *et al.*, 1972) there was no difference in label between cells in B areas and those in T areas.

TABLE 5. The fate of an intravenous injection of an *in vitro* labelled single cell suspension of spleen cells from donors primed against BSA

	Number of rats	Red pulp	Perifollicular area	Periphery of follicle	Thymus-dependent area
Number of [^{3}H]adenosine-labelled cells* per unit area of recipient spleen†	4	0·31 ± 0·47	0·30 ± 0·47	4·22 ± 2·86	4·05 ± 2·36
Percentage distribution of [^{3}H]adenosine-labelled cells* in recipient spleen	4	5·1 ± 2·60	8·1 ± 1·8	44·6 ± 6·3	42·2 ± 7·7

* Autoradiographs were exposed for 9 weeks.

† All recipients received 8×10^7 [^{3}H]adenosine-labelled spleen cells i.v. and were killed 24 hr later.

Labelled cells in the blood (Table 2)

Most cells would travel from the donor to recipient spleen in the circulating blood and, indeed, there were labelled small, medium and large lymphocytes in the blood of all spleen graft recipients taken immediately before killing. The number of labelled cells in the blood (Table 2) would appear to reflect the donor rather than recipient spleen impression smears (Table 2), although due allowances must be made for the difference in exposure time (blood 13 weeks; impression smears 5 weeks).

Labelled cells in thoracic duct lymph (*Table* 2)

A thoracic duct cannula was inserted into six out of nine rats but in only one animal (L5) did lymph flow for longer than a few hours. The difficulties in prolonging lymph drainage were attributed to persistent clotting in the cannula secondary to an increase of blood in the lymph following transplantation. All samples of lymph taken contained a small percentage of labelled cells. The one successful long-term cannulation (Rat L5) was started 48 hr after transplant and continued for 3 days. Between 2–4% of lymphocytes collected from the thoracic duct were indeed labelled, although in general the observations on the content of labelled cells in thoracic duct lymph were too limited for any meaningful conclusions.

The distribution of labelled cells in cervical lymph nodes (*Table* 6)

Labelled cells were found in all areas of all the lymph nodes examined. Unit area counts were made in a representative lymph node of each recipient, the cervical. As there were

TABLE 6. Number of labelled cells* per unit area of recipient cervical lymph nodes

Animal serial number	Post-isotope	Post-grafting	Nodular area (mean ± s.d.)	Thymus-dependent area (mean ± s.d.)	Medulla (mean ± s.d.)
L2	3	1	1·8 ± 0·8	2·2 ± 0·5	n.d.
L9	3	1	4·0 ± 1·2	2·4 ± 1·3	2·7 ± 1·6
L6	6	3	3·0 ± 0·7	3·7 ± 2·4	2·7 ± 0·6
L7	6	3	5·0 ± 1·4	4·3 ± 2·3	3·0 ± 1·5
L1	5	4	3·2 ± 1·1	3·3 ± 1·7	1·0 ± 0·0
L4	8	4	2·3 ± 1·0	2·3 ± 0·8	1·8 ± 1·0
L5	6	5	3·5 ± 0·8	3·6 ± 1·4	1·2 ± 0·5

n.d. = Not determined

* Autoradiographs were exposed for 10½ weeks.

very similar numbers of cells in both the TDA (thymus-dependent area) and in the nodular areas, it would appear that approximately equal numbers of labelled T and B lymphocytes must have entered the nodes from the blood. As in the recipient spleen there was no increase in numbers of labelled cells with time after spleen grafting. The actual number of cells per unit area were, however, slightly lower than the equivalent T (TDA) and B (periphery of the follicle) in the recipient spleens.

Gut-associated lymphoid tissues

As in previous studies in the migration of intravenously injected lymphoid cells to the gut (Parrott and Ferguson, 1974) three sites were examined for the presence of labelled cells; Peyer's patches, the lamina propria of villi in the small bowel, and the epithelial layer of villi.

Peyer's patches (*Table* 7)

The numbers of labelled small lymphocytes found in T and B areas were very similar to those (Table 6) in autoradiographs of cervical lymph nodes exposed for the same length

TABLE 7. Number of labelled cells per unit area of recipient Peyer's patches

Animal serial number	Post-isotope	Post-grafting	Nodular area			Thymus-dependent area		
			5 weeks* (mean ± s.d.)	10½ weeks* (mean ± s.d.)	17½ weeks* (mean ± s.d.)	5 weeks* (mean ± s.d.)	10½ weeks* (mean ± s.d.)	17½ weeks* (mean ± s.d.)
L9	3	1	2·6 ± 0·9	3·8 ± 1·3	5·2 ± 0·8	2·3 ± 1·0	4·8 ± 0·5	4·6 ± 1·7
L6	6	3	1·3 ± 1·0	2·0 ± 0·8	2·8 ± 1·3	1·5 ± 0·6	2·5 ± 1·2	2·3 ± 1·1
L7	6	3	4·0 ± 1·4	5·0 ± 0·6	5·0 ± 1·0	3·5 ± 1·8	6·0 ± 2·0	4·1 ± 2·8
L1	5	4	1·3 ± 0·5	3·8 ± 1·1	3·8 ± 0·5	0·8 ± 0·4	2·9 ± 1·0	3·2 ± 1·1
L4	8	4	1·9 ± 0·7	2·6 ± 1·5	2·2 ± 1·2	1·5 ± 0·8	1·7 ± 1·2	1·9 ± 0·9
L5	6	5	2·5 ± 1·0	5·5 ± 1·0	3·8 ± 1·0	1·4 ± 0·9	3·5 ± 1·8	3·6 ± 0·8

* Exposure time of autoradiographs.

of time. Counts were made in autoradiographs of Peyer's patch tissue for three different exposure times, 5 weeks, 10½ weeks, 17½ weeks. In general, there was a progressive increase in numbers with greater exposure times although only some of the differences between 5 weeks and 10½ weeks were significant and more between 10½ and 17½ weeks. Particular attention was given to what has been termed the dome area of Peyer's patches since recent studies have shown that not only is the subepithelium a thymus-dependent area (Parrott & Ferguson, 1974) but that labelled thymus cells in newborn mice have been seen apparently in transit, in the epithelial layer itself (Waksman, 1973). In only one rat (L7) did one or two labelled cells appear in or very near the epithelium of the dome in two sections of Peyer's patches (Fig. 6).

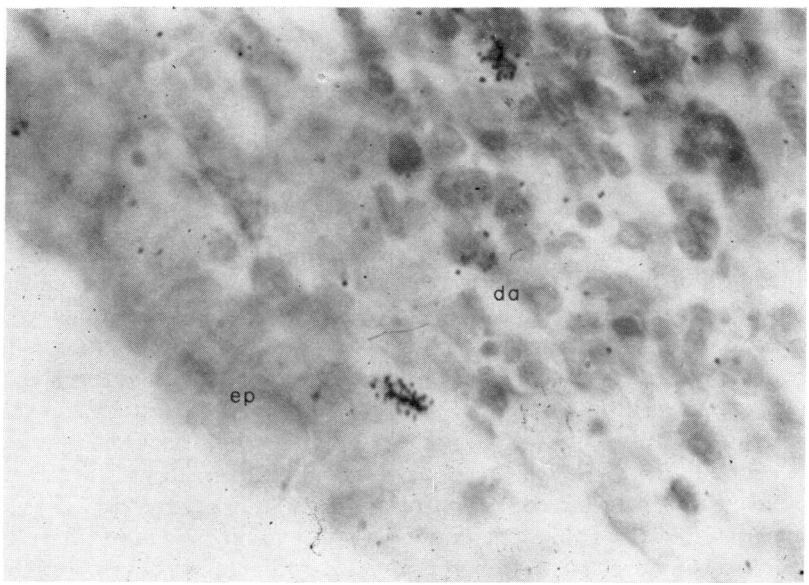

FIG. 6. Peyer's patch from recipient (L7) killed 3 days after transplant. Labelled cells in or very close to epithelium (ep) over dome area (da) of Peyer's patch. The exposure time was 10½ weeks. (Magnification × 665.)

Lamina propria

Although studies with dissociated spleen cells labelled with [^{3}H]adenosine or [^{3}H]uridine have shown these cells to migrate to Peyer's patches, very rarely do they enter the lamina propria except in villi close to Peyer's patches (Parrott & Ferguson, 1974). It was very surprising, therefore, to find appreciable numbers of labelled cells from [^{3}H]thymidine-labelled spleen grafts, some differentiated into plasma cells, in the lamina propria of the recipient's gut (Table 8). Moreover, it would appear that these cells are derived from rapidly dividing precursors, since an increase in exposure times increased substantially the number of labelled cells found. Careful examination of the epithelial layers again revealed the presence of labelled cells in one rat (L7), the selfsame animal in which labelled cells were found in the dome epithelium of Peyer's patches. It should be emphasized that insufficient

numbers were found to be certain that such cells had passed the basement membrane or indeed that they were lymphocytes comparable to those cells usually described as intra-epithelial lymphocytes.

TABLE 8. Number of labelled cells per hundred villi in lamina propria of small bowel

Animal serial number	Post-isotope	Post-grafting	5 weeks*	10½ weeks*	17½ weeks*
L9	3	1	2·9	2·6	13·8
L6	6	3	0	22·8	44·4
L7	6	3	12·0	4·8	7·1
L1	5	4	1·3	0	25·2
L4	8	4	1·0	8·9	24·2
L5	6	5	19·1	35·9	119·2

* Exposure time of autoradiographs.

DISCUSSION

The main intention of the present experiments was to determine which of the various cell types in addition to the known recirculatory T and B lymphocytes present in a whole spleen transplant were capable of spontaneous rather than the enforced migration which many of the cells in a dissociated suspension of a tissue may undergo after an intravenous injection. Labelled cells were present in all areas of the donor spleen at the time of transplantation, plasma cells, lymphocytes and blast cells in the red pulp; lymphocytes and blast cells in the white pulp; light and heavily labelled cells in germinal centres. All will be the progeny of cells which have divided during the period of administration of isotope 1–2 weeks before grafting. Some will be continuing differentiation and division, others will have ceased. In the lymphoid tissue of the recipient there were almost equal numbers of labelled lymphocytes in both B and T areas but there were also significant numbers of labelled cells at two other unexpected sites, the red pulp of the recipient spleen and the lamina propria of the gut. The persistence of labelled lymphocytes in the perifollicular area and red pulp of the recipient spleen at all times after grafting was in contrast to our previous experience with labelled suspensions of spleen cells which, like thoracic duct lymphocytes, accumulate in these sites immediately after i.v. injection (Ford, 1969b; Parrott & Sousa, 1971) but are thereafter found only in the B and T areas of the white pulp. It is true that when suspensions of spleen cells labelled *in vivo* with [^{3}H]thymidine were used (Parrott & Sousa, 1971) rather than *in vitro* [^{3}H]adenosine or [^{3}H]uridine there were larger numbers of cells than usual in the red pulp but within 1–2 days these cells assumed the familiar distribution pattern with the majority in the white pulp. It could be argued that the labelled cells in the red pulp of the recipient spleen were not cells which had migrated directly from the red pulp of the donor but were derived from small lymphocytes which had entered the white pulp of the recipient differentiated and then moved across to the red pulp. This, however, is a process which takes at least 4–5 days and requires the presence of intravenous antigen (Ellis & Gowans, 1973) and there were already heavily labelled cells in the red pulp of the recipient at 1 day after

grafting. The identity of the labelled cells in the recipient spleen is not known; it would seem likely that they are precursors of antibody-forming cells although very few differentiated into obvious plasma cells during the period of the experiment. One conclusion from these experiments is, therefore, that potentially mobile lymphocytes are present in the red pulp of the spleen. These cells may not, however, be present in the spleen at all times, but only develop in the course of an immune response, in this particular case the rejection of a skin allograft. These experiments confirm previous observations that nests of plasma cells and germinal centres are amongst the relatively sessile populations in the spleen. It would appear that the donor spleen also contained precursors of cells with the capacity to home to the gut. Cells with this capacity have previously only been found in thoracic duct lymph (Gowans & Knight, 1964; Hall, Parry & Smith, 1972) and mesenteric lymph node (Parrott & Ferguson, 1974). In the present experiments there was a progressive accumulation of labelled lymphocytes and plasma cells which did not reach a maximum until 5 days. This is one reason why they were not detected in previous studies with spleen cell inocula (Parrott & Ferguson, 1974) which were limited to 2 days after the infusion of cells. It also indicates that those cells in the spleen which were destined for the lamina propria were probably at an earlier stage of differentiation than those in thoracic duct lymph which home to the gut within 4 hr of injection (Hall *et al.*, 1972).

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